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Author	Lutu, Wiggins Zakayo
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THE EFFECT OF REPEATED RHIPICEPHALUS APPENDICULATUS
NEUMANN 1901, (IXODIDAE) INFESTATIONS ON THE HEALTH OF
CATTLE.

WIGGINS ZAKAYO LUTU

Dip.Vet.Sc. (Makerere), B.V.Sc. (E. Africa), Dip.T.V.M., M.Sc.
(Edinburgh)

Ph.D.

Department of Tropical Animal Health, Centre for Tropical
Veterinary Medicine, University of Edinburgh.

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DECLARATION

I declare that this thesis has been composed by me and is entirely my own work.

W.Z. Lutu.

Dedication

To Mrs Susan Valentine for her invaluable contribution
and my children, Patrick, Onesmus and Judith for their
patience, tolerance and support.

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GLOSSARY

- Acantholytic - dissolution of the intercellular bridges in the prickle-cell layer of the epidermis; it is one of the mechanisms of formation of the intraepidermal vesicles.
- Adipsia - absence of thirst, or abnormal avoidance of drinking.
- Alopecia - [a disease in which the hair falls out]. loss of hair from skin areas where it normally is present - associated with tick feeding effects.
- Anisocytosis - presence in the blood of erythrocytes showing excessive variation in size.
- Anorexia - lack or loss of appetite for food.
- Anoxia - reduction of oxygen in body tissues below physiologic levels. (stagnant anoxia, anoxia resulting from inadequate blood flow through capillaries with resultant abnormal oxygen extraction and low tissue oxygen tension).
- Anuria (anuresis) - absence of excretion of urine from the body; possibly of renal or suppressive origin in the affected guinea pigs. (renal a., a failure of urinary secretion by the kidney in presence of adequate filtration pressure in the glomeruli and patency

of the ureters. Suppressive a., failure of secretion of urine in the kidneys).

- Bleb - a small circumscribed elevation of the cuticle (epidermis) usually containing serum: a blister (vesicle). Different manifestations as provoked by tick feeding are described in C.5.1.8.
- Canker - inflammation of the lining of the external ear associated with tick and fly maggot infestation.
- Cellulitis - diffuse and subcutaneous inflammation of connective tissue (accumulation of sanguineous fluid) in areas infested with Rhipicephalus appendiculatus ticks.
- Dermatitis - inflammation of the skin in areas infested with ticks: primary irritant dermatitis and allergic dermatitis.
- Diapedesis - the passage of blood cells through capillary walls into the tissues without detectable gross lesions.
- Dyspnoea - difficult or laboured breathing, characteristics manifested vary depending on cause or part of respiratory system affected.

- Eczema - a superficial inflammatory process involving primarily the epidermis, characterised early by redness, itchiness, minute papules and vesicles, weeping, oozing and crusting and later by scaling. It is not a disease entity. More severe in sensitized animals (allergic).
- Erythema - abnormal redness of the skin due to capillary congestion of localised or generalised diffuse nature caused by tick feeding.
- Exudate - material, such as fluid, cells or cellular debris, which has escaped from blood vessels and has been deposited in tissues or on tissue surfaces, as result of inflammation - characterised by high content of protein, cells or solid materials derived from cells.
- Fasculation - a small local contraction of muscles, visible through the skin, representing a spontaneous discharge of a number of fibers innervated by a single motor nerve filament.
- Hyperkeratosis - thickening of the corneous layer of the skin (as result of persistent irritation from tick feeding.)

- Instar - a stage in the life of an insect or other arthropod between two successive moults.
- Lymphadenopathy - abnormal enlargement of lymph nodes.
- Oliguria - reduced excretion of urine.
- Otoacariasis - infestation of the ears with ticks (Acarina) and resultant pathological changes.
- Pathognomonic - specifically distinctive or characteristic of a disease or pathologic condition; a symptom or sign on which a diagnosis can be made.
- Pityriasis - skin epidermal reaction marked by formation and desquamation of branny scales as result of irritation caused by tick feeding.
- Poikilocytosis - presence in the blood of erythrocytes showing abnormal variation in shape.
- Polychromasia - variation in the haemoglobin content of the erythrocytes of the blood - revealed by abnormal variety or change of colours on staining (both acid and basic dyes).
- Pruritis - localised (or generalised) itching due to irritation of sensory nerve endings from pharmacological agents released by tick feeding.

Pyrexia	-	elevation of body temperature to an abnormal level.
Reticulocytosis	-	abnormal presence in blood of non-nucleated erythrocytes that, when ^{supravitaly} stained with new methylene blue, present one or more granules or a diffuse network of fibrils.
Sclerotisation	-	pathological hardening of tissue produced by overgrowth of fibrous tissue and other changes.
Tachycardia	-	excessive rapidity in action of heart rate.
Tachypnoea	-	increased rate of respiration - marked by quick and shallow breathing.
Tenesmus	-	a painful and distressing but ineffectual urge (straining) to evacuate the rectum.
Thrombocytopenia	-	decrease in the number of blood platelets.
Transudate	-	fluid substance which has passed through a membrane (capillary), sometimes as a result of inflammation, characterised by high fluidity and low content of protein, cells, or solid materials derived from cells.
Urticaria	-	a vascular reaction of skin marked by the transient appearance of

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smooth, slightly elevated patches
(wheals). The eruptions rarely
last longer than two days -
associated with hypersensitivity
to tick secretions.

ABSTRACT

THE EFFECT OF REPEATED RHIPICEPHALUS APPENDICULATUS INFESTATIONS ON THE HEALTH OF CATTLE.

Clinical-pathological responses were investigated in Bos taurus and guinea pigs infested with ticks from a laboratory colony. Infestation regimes involved three instars individually or concurrently in five separate groups with five calves each except one. Repeated infestations were made over calf ears and guinea pig trunks. Systematic clinical examinations, haematological and biochemical estimations, and autopsies were undertaken. Tick feeding and oviposition performance was monitored. Demonstration and corroboration of the findings was effected by further experiments involving cutaneous anaphylaxis tests and use of antihistamines against induced responses.

Prominent responses occurred in the integumentary, musculo-skeletal, cardiovascular, lymphatic and nervous systems. Significant findings were: inflammation, exudations and encrustations, hypersensitivity reactions; extreme emaciation, prostration and death; mono and diphasic pyrexia, congestion, anaemia, excessive mucosal secretions, significant haematological and biochemical differences between calf groups; lymph node enlargement and abscessation; grooming associated with pruritis. Autopsies confirmed clinical manifestations. Ticks were adversely affected, especially larvae. Cutaneous anaphylaxis, antihistaminic effects, vivid guinea pig

circulatory disturbance were demonstrated.

Rhipicephalus appendiculatus infestations caused serious effects culminating in death; and exsanguination and intoxication are strongly incriminated. Therefore field studies should consider the role of ticks. But the precise mechanism causing debility is not clear, thus a critical study of the factors causing loss in productivity is indicated.

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

Ticks are responsible for maintenance and propagation of many human and animal diseases. It is the losses in domestic cattle caused by these diseases, as much as the direct action of the ticks themselves, which make the tick infestation so significant for the stock-owner. With disease, the losses may be sudden and tangible, with the carcasses of animals as evidence, whereas the debilitating effect of tick infestation, resulting in unthriftiness, lower yields and deaths is less clearly seen. The dramatic and severe losses in livestock caused by tick-borne diseases do overshadow the other serious injuries inflicted during heavy infestations which concurrently or separately can increase the host susceptibility to diseases. However, it is recognised that ticks can cause severe adverse effects without disease transmission (Theiler, 1921). Field observations where the two phenomena are concurrent seldom clarify the specific role played by tick effects in the overall host response. The unproven assertion on the aggravating effect of Rhipicephalus appendiculatus infestation on tick-borne syndromes was made by Thomas & Neitz (1958).

The physical effects cause blood loss, irritation,

disturbed foraging, tissue wounds and predisposition to secondary infections. However, mild repeated infestations induce acquired resistance in the host manifested in various forms. Tick paralysis or toxicosis, is quite distinct from tick-borne disease in that the causal factor is a toxic substance and not a disease organism (Gregson, 1973). Several different mammals may be paralysed by a single tick species, e.g. Ixodes holocyclus, and several tick species can cause paralysis in a particular host. Paralysis which can commonly affect cattle in North America infested with Dermacentor andersoni is caused by toxins contained in the salivary secretions injected in/^{to}the host during tick feeding. Toxicoses affecting cattle are represented by sweating sickness and rhipicephaline toxicosis as described in South Africa (Neitz, 1962).

It is well recognised that different host species respond differently to the same or different parasitic stimuli, thus results obtained from a particular host-parasite association cannot generally apply to others of different composition. This certainly applies to results obtained from tick and laboratory animal association which may not necessarily be comparable with associations between ticks and farm animals. In this connection, considerable information on the effects of Boophilus microplus infestations in cattle in Australia has been well documented (Riek, 1956, 1962; Tatchell & Moorhouse, 1968).

Tick-borne diseases assume their greatest economic importance in the tropics, where they are both wide-spread and numerous (Ann. Rep., Kenya Vet. Dept., 1970). Consequently there is still a need for further critical studies on the role of various uninfected tick species themselves in the pathogenesis of host responses commonly associated with farm animal diseases. Most field investigations seem not to distinguish between direct tick effects and those caused by the pathogens transmitted because of the difficulty of distinguishing non-infected ticks in such circumstances.

Rhipicephalus appendiculatus has been reckoned one of the ticks of greatest importance in Africa, South of the Sahara, where it is the principal vector of East Coast fever caused by the protozoan, Theileria parva, in addition to other diseases (du Toit & Theiler, 1964). There is a paucity of data on the specific clinical-pathological effects caused by disease-free Rhipicephalus appendiculatus ticks on the bovine host (van Rensburg, 1959). Nonetheless, a considerable amount of work has been carried out with other species, e.g. Boophilus spp. (O'Kelly & Seifert, 1970), Amblyomma spp. (Williams et al., 1978). However, the responses to these ticks cannot empirically apply to R. appendiculatus since work in Australia (Riek, 1956; Tatchell & Binnington, 1973) and other countries (Neitz et al., 1969; Howell, 1966) has shown considerable variation in host reactions even to one tick species. The cattle infected naturally with

tick-borne pathogens show many signs which are inconsistent with the common clinical syndromes of such diseases.

There is therefore need for further investigation of the host-parasite relationship with regard to R. appendiculatus.

1.2. LITERATURE REVIEW

1.2.1. GENERAL

Theiler (1921) observed that large tick numbers caused enormous damage, such as exsanguination, irritation, and possible death without even transmitting disease. Experimental exposure to Boophilus decoloratus induced acute fatal anaemia in a horse and a heifer both of which developed extreme pallor. Death was attributed to exsanguination caused by massive infestation. Within three days of first engorgement fourteen pounds of female ticks had detached from the horse and it was reckoned this weight only represented about half of the ticks which had engorged on the animal. A similar observation was made on the heifer. Philip, Jellison & Wilkins (1935) and Thomas & Neitz (1958) observed signs in their studies which were not consistent with the transmissible diseases. The manifestations associated with large tick numbers were physiological and pathological in nature, e.g. affected gait, temperature, dyspnoea, desultory feeding, restlessness, significant drop in haemoglobin levels. The severe reactions culminated in prostration and rapid death (see glossary for clinical terms). Philip et al., (1935) found deticking in the early stages was followed by rapid and complete recovery. The tick effects exacerbated the disease syndromes which terminated fatally and where precise evidence for their role in the syndromes was lacking, such effects as anaemia were presumed contributory.

evidence was lacking. Pavlovsky & Alfeyeva (1941) observed that ticks also exhausted the animals and contended that the local reaction to tick bites had not been studied enough. They suggested consideration of factors like devitalisation of skin integrity, manner of attachment and reaction to bite from non-infected ticks. Thus violation of the skin defensive barriers enabled pathogens to gain entry into the body. In addition lysis of elastic fibres and formation of pitted scars reduced the quality of the industrial value of the skin.

Cowdry & Danks (1933) cited Cleland (1910) who noted exudation from tick bites which they correlated with vascular and blood cell changes in histological studies. They presumed ^{that} changes were brought on by salivary gland secretion though no record of previous study of such activity could be traced. Since then extensive studies have been carried out in different tick-host systems on the local reactions to primary and secondary infestations (Tatchell & Moorhouse, 1968; Tatchell & Binnington, 1973; Kemp, 1978).

Wood (1968) reviewed the resultant effects of blood sucking activities of heavy infestation and suggested the tick mouthparts provoked inflammation and erythematous nodule formation and pruritis which lead to traumatization and secondary complications, such as ^{bacterial} / infections. He concluded that the typical arthropod parasitic dermatoses were usually due to combination of

primary and secondary responses, and thus cited sweating sickness in cattle and Queensland itch in horses associated with toxins from Hyalomma truncutum and Boophilus microplus respectively. Tatchell (1969a) speculated that much of the tissue destruction noted with B. microplus might be a result of an inflammatory response to tick antigens and entirely co-incidental to a more direct attack on the host vascular system by the tick saliva. He suggested the specific responses to blood sucking arthropods should be considered in general as they can also be mediated through pharmacological agents released from saliva-host tissue reactions. It was thus argued that there was development of antigen-antibody complexes, which were themselves highly toxic to host tissues. Toxic effects would release histamine and heparin leading to circulatory disturbances. Riek (1955, 1962) demonstrated elevated histamine levels on exposure of sensitized host to further infestation. Tissue changes attributable to vascular derangement were demonstrated during immunological studies by Riek (1956, 1962) and Willadsen et al., (1978). In reference to Gregson (1960) and Balashov (1965) Tatchell (1969b) asserted that the non-mechanical vascular damage occurred early on during attachment evoking a free flow of blood. Citing the study by Berlin (1957) on the pathological effect of tick secretion on arterial endothelium he commented that the effects induced pharmacological activity and

need not involve lytic action. This view has been upheld by other studies (Tatchell & Moorhouse, 1968, 1970; Tatchell & Binnington, 1973).

However, Moorhouse (1968) induced lysis of conchal cartilage in guinea pigs infested with Ixodes sp. Tatchell & Moorhouse (1968) reckoned that the feeding lesion was a result of host-parasite interaction whereby the specific vascular damage possibly occurred through tick saliva and tissue damage through host response. The composition and effects of the salivary secretion in different ticks associated with specific effects in tissue have been investigated by the same authors and others (Howell et al., 1975; Schleger et al., 1976). It was further argued that the damaged tissues liberated pharmacologically active substances which could equally cause adverse systemic effects. Postulations on the basis of local responses indicated Arthus-type or anaphylaxis reaction, demonstrated by preponderance of neutrophils along with necrosis of tissues and vascular damage leading to haemorrhage. Tatchell & Moorhouse (1968, 1970) observed inflammatory changes (erythema, oedema, haemorrhage) which were attributed to saliva action mediated through collagen destruction and vascular dilatation and leakage. Thomas & Neitz (1958) had asserted that the pronounced oedema of the head and ears preceding patent disease manifestations was suggestive of massive tick toxin liberation. Tatchell & Binnington

(1973) with the help of Dr. D.H. Kemp, demonstrated pharmacological activity in tick saliva which provoked dilatation of dermal capillaries and seepage of 'plasma'. Kemp inoculated saliva intradermally into susceptible calves and its effect on capillary endothelium was demonstrated by leakage of $^{125}_{51}\text{I}$ (radioactive iodine) or Evans Blue-labelled albumin. Similar immediate vascular changes (capillary permeability and dilatation) were also observed on infestation with Boophilus microplus larvae in cattle (Tatchell & Moorhouse, 1968). Howell (1966) using Ornithodoros savignyi salivary secretion noted the importance of saliva as a mediator of physiological-pathological manifestations unrelated to infections in normal animals. He thus presumed that the unrelated signs were caused by "toxins" contained in the complex salivary secretions. Sudden deaths overnight in bovines attacked by Ornithodoros sp. ticks were indicative of highly patent toxin. Neitz et al., (1969) demonstrated a lethal factor in the protein fraction of O. savignyi salivary secretion in mice in which the effects were mediated through proteolytic and cholinesterase activities. Howell et al., (1975) associated death in animals with toxic constituents of oral secretion besides the effects of exsanguination.

Riek (1957b) and Seebeck et al., (1971) suggested that the slow recovery, despite cessation of infestation and adequate feeding in heavily infested animals in poor condition, was an indication of prolonged toxic effects of

salivary secretion. However, such effects were thought transient in the absence of concomitant infections. Macleod (1933) incriminated ticks for depressed productivity, unthriftiness, stunted growth and deaths in lambs. Thomas & Neitz (1958) suggested that the rhipicephaline toxicosis associated with relapses of various haematogenous parasitic infections influenced the severity of clinical syndrome exhibited by cattle exposed to massive tick infestation. The relapses were presumably caused by a suppression on the reticuloendothelial system defensive mechanism by the toxic agent. Despite the incomplete and fragmentary nature of the accumulated evidence they advanced certain preliminary conclusions and conjectures implicating direct tick effects. On the other hand the frequent occurrence of such an extreme lethal condition in the field was discounted though the resultant unthriftiness, loss of condition and retarded growth were considered important. The conclusions/conjectures have been exploited by other studies involving different types of ticks and animals.

Riek (1957b) inoculated tissue extracts from different ticks into laboratory animals to demonstrate intoxication but the response was not uniform in different hosts and extracts from some of the ticks were ineffective. Howell (1966), in agreement with van Rensburg (1959), revealed varied susceptibility in different hosts to secretions of even one kind of tick. The varied susceptibility could also be attributed to saliva

composition in different ticks as demonstrated by Neitz et al., (1969) and Tatchell & Binnington (1973). van Rensburg concluded that the extensive damage induced by Rhipicephalus spp. in the bovine could be caused by a cytotoxic substance as reflected in lymph node changes. However, these findings were complicated by limited accuracy, a premune state and concomitant disease relapses and lack of distinct evidence for direct cytotoxicosis. Nevertheless, much of the observations made by Thomas & Neitz (1958) on rhipicephaline toxicosis were reproduced, thus their data was described as irrefutable evidence of potent toxin injected by R. appendiculatus. On account of varied susceptibility van Rensburg (1959) recommended that the diagnosis of clinical toxicosis should be based on a herd unit instead of the individual animal which could suffer an obscure debilitating disorder. In support of these observations, Neitz (1962) stated that toxicosis, caused by a leucocytotropic toxin, frequently caused severe losses in cattle exposed to massive R. appendiculatus infestation in the bushveld and lowveld in South Africa.

Little (1963) claimed the direct losses from tick infestation, comprising losses in beef and milk production, both from deaths and tick worry had not been accurately defined. Seifert (1971b), in agreement, demonstrated significant retardation in growth rate in protractedly infested cattle and found highly significant negative

correlation between tick numbers and growth rate. Little (1963), and Johnston & Haydock (1969) held a view that the recorded deaths were the result of deprivation from tick effect in consort with other contributory factors, such as nutrition. Heavy tick infestations reduced feed intake (O'Kelly et al., 1971; Seebeck et al., 1971), caused retarded growth or weight gain losses (Francis, 1960; O'Kelly & Seifert, 1970; O'Kelly & Spiers, 1976; Williams et al., 1977, 1978). The failure to gain weight could be attributed mainly to reduced feed intake or to joint 'anorectic' and specific effects (Seebeck et al., 1971, O'Kelly & Seifert, 1970). The specific effects upon the host metabolism could arise from toxins secreted by tick salivary glands (Riek, 1957b, van Rensburg, 1959; O'Kelly et al., 1971). However, high nutritional status may have a profound ameliorating influence on the susceptibility to tick effects but this could be eliminated by very heavy infestation as revealed in the above and other studies (O'Kelly & Seifert, 1970; Gladney et al., 1973).
(1970)
O'Kelly & Seifert/ thus stated that even adequate diet could not counterbalance the depression in growth rate and blood composition caused by heavy tick burdens.

duToit & Theiler (1964) in agreement with Hitchcock (1955) and Little (1963) commented on the serious clinical effects and the enormous cost incurred in control. Besides exsanguination animals lost condition

through tick worry and toxicosis and inflicted wounds. The heavy infestation caused toxicosis and marked decrease in resistance to tick-borne diseases. The bite wounds facilitated severe secondary local and deep-seated tissue complications, such as suppuration and abscessation, necrosis and sloughing and myiasis, which might end in death or deformities. In conformity with Clifford (1954) Rhipicephalus appendiculatus was rated by du Toit & Theiler among the important tick species, thus stating, '...perhaps the most harmful to stock, for apart from the diseases it transmits it can also cause deep-seated abscesses, leading to crumpling or even the loss of ears, as also to severe injury of the udder and tail. Heavy infestations may also lead to tick toxicosis, resulting in marked falling off in condition of cattle and a marked decrease in resistance to tick-borne diseases'.

1.2.2. PERFORMANCE OF TICKS

Behaviour of ticks before feeding influences the rate of attachment (Arthur, 1973). Cowdry & Danks (1933) and Branagan (1974) observed Rhipicephalus appendiculatus and found some ticks attached immediately while others spent a day crawling around, occasionally making several abortive attempts to attach, before a final place was selected. Similar findings have been recorded in other

tick species, especially Boophilus microplus (Hitchcock, 1955; Tatchell & Moorhouse, 1968; Kemp & Koudstaal, 1971). It is worth noting that the greatest bulk of work has been done on B. microplus, a one-host tick. Roberts (1968b, 1971), Bennett (1969, 1975), Kemp et al., (1976) Koudstaal et al., (1978) reckoned no successful larval attachment could occur later than 24 hours after application. However, Hitchcock (1955) and Riek (1959) reported successful attachment after much longer delay. Branagan (1974) and A.R. Walker (personal communication) found that the adult R. appendiculatus ticks applied on rabbits sometimes take 3 days to attach. Sex ratio does not influence the rate of attachment in R. appendiculatus (Irvin et al., 1973; Branagan, 1974).

The reason for the delayed attachment was obscure, though Cowdry & Danks (1933) had suggested blood supply and secretion of cutaneous gland factors. Irvin et al., (1973) demonstrated delayed attachment in R. appendiculatus nymphs when more than 4,000 ticks were applied. The speed of attachment could be influenced by age (Branagan, 1974; Riek, 1962; Tukahirwa, 1976) and environmental temperature (Irvin et al.,¹⁹⁷³ Roberts, 1971). Hoogstraal (1956) commented on the considerable variation in tick appearance and morphological details which affected size, robustness and certain physical characteristics. Such factors associated were crowding on hosts, welfare of immature stages and availability of suitable hosts.

Host suitability has been studied by various workers and for some unknown reason, it was found that calves carried much lower infestations than adults under the same environment (Barnett & Bailey, 1950; Utech et al., 1978a; Paperna, 1972; R. Newson, pers. comm.). Yeoman (1966) thus considered calves an unsuitable indicator for the degree of infestation in an area. O'Kelly & Spiers (1976) suggested colostral antibody constituted an important resistance component. Baker & Ducasse (1968) incriminated goats as an important R. appendiculatus reservoir for cattle relative to diseases, but McCulloch et al., (1968) and Branagan (1974) found the goat a less efficient host. Differences in performance of ticks fed on different hosts was demonstrated by Riek (1959), Branagan (1974), du Toit & Theiler (1964) and Irvin et al., (1973) whereby cattle were rated as most suitable and guinea pigs as unsatisfactory. Breed susceptibility could hamper the viability of ticks (O'Kelly & Spiers, 1976; Riek, 1962; Wilkinson, 1962; Utech, Wharton & Kerr, 1978).

The resistance state of hosts with or without gross tissue reactions impedes successful attachment (Munro Hull, cited by Francis, 1966; Trager, 1939a; Bailey, 1960; Riek, 1962; Roberts, 1968c; Allen, 1973; Kemp et al., 1976). Munro Hull is claimed to be the first to bring the phenomenon under public notice in Australia when he reported such cattle which also developed skin

exudation. He further contended that resistance could be transmitted by vaccination but Johnston & Bancroft (1918) (cited by Francis, 1966) were unable to confirm this claim. Nevertheless, his observations have since been extensively investigated. Moorhouse & Tatchell (1966) asserted that the attachment process was mechanical without provoking inimical reaction in the host and the resistance was acquired in the succeeding phases of feeding. However, Gregson (1970) reported adverse host tissue reaction. Thus, Bailey (1960) recommended use of rabbits only once for successful tick performance.

Riek (1956) noted that increasing the number of larvae beyond 100,000 caused prolongation of the period of both maturity and dropping of engorged B. microplus females. Similarly Irvin et al., (1973) demonstrated an extended engorgement period and reduced weight in R. appendiculatus nymphs applied to cattle in large numbers compared with ticks fed in smaller numbers. Observations made by Sutherst et al., (1978) conform to these findings. In contrast, Branagan (1969) found significantly reduced mean time to engorgement for larger infestations which was attributed to clustering propensities associated with acquisition of resistance. It was noted that larger numbers had an accelerating effect. This was not consistent with the stipulation made by Lees (1952) on

cuticular growth during feeding. The cuticle requires time to develop sufficiently in order to accommodate the sudden increase in size as the tick rapidly engorges. Irvin et al., (1973), reckoned that the clustering behaviour of adult Rhipicephalus appendiculatus described by Yeoman & Walker (1967) was a prerequisite for adequate feeding. Thus a figure of 2000 nymphs per ear was considered optimal for improved production of infected ticks in the East Coast fever transmission research. Sutherst et al., (1973) found that large infestations were accompanied by reduced larval survival rates. This was associated partly with a competition effect in the tick population which functions as a density-dependent mortality factor. However, later they found that mortality on susceptible cattle was largely independent of density (Sutherst et al., 1978).

The environmental factors, such as temperature, influenced the successful engorgement as shown by Bailey (1960), Stampa (1959), Branagan (1974), Kemp et al., (1976). But Hitchcock (1955) did not find the temperature effect significant in the parasitic life cycle of B. microplus. Purnell et al., (1972) found that withholding mating deterred or prolonged the engorgement of female R. appendiculatus which corroborates studies on the influence of mating on the repletion of female ticks carried out by Norris (1954). However, Irvin et al., (1973) demonstrated that feeding during the first four days was not dependent on the presence of males. The range of engorgement periods on primary exposure to R. appendiculatus ticks in both rabbits and

cattle were comparable though mean weights were heavier in cattle fed ticks as revealed by Irvin et al., (1973) and Branagan (1974). Branagan demonstrated significantly prolonged engorgement period, reduced numbers and weight in surviving ticks during repetitive rhipicephaline infestations in rabbits which reacted severely. However, such responses were not found in the ticks used for East Coast fever transmission experiments in cattle and further studies on the ticks were not done for lack of suitable cattle. Nevertheless, other workers, like, Trager (1939b), Riek (1962), Bailey (1960), Allen (1973), Doube & Kemp (1975), Hewetson (1971) found repetitive exposures in cattle and rabbits induced much severer restriction on tick performance. Theiler (1921) recorded fairly constant engorgement periods in all R. appendiculatus instars in cattle which was later supported by du Toit & Theiler (1964) who reckoned adults were the quickest feeders. Musatov (1978) studied natural and artificial infestation associated with four tick genera in cattle, sheep and rabbits and concluded that the host reactions to ticks, their attachment and feeding, varied according to the species and immune status of host and the type of tick. He observed necrotic dermatitis accompanied by exudation around attachment points during secondary attack which was attributed to a severe immune reaction.

Hypersensitivity, in the form of blebs, hindered engorgement either through premature detachment or shedding

together with degenerate bleb pellicle (Branagan, 1974; Trager, 1939a; Riek, 1956; Allen, 1973).

Riek (1962) described the bleb grossly as 'a small blister filled with clear fluid later becoming a yellowish pustule which on breaking forms a scab and on healing leaves a pitted scar'. Tatchell & Moorhouse (1968) described it as an intraepidermal acantholytic lesion which was comparable to the reaction provoked by cantharidin embrocation. Tatchell & Moorhouse (1968) affirmed the interference of host responses with successful tick feeding, causing detachment within two hours and desertion of the area by 24 hours as the surface became encrusted. These responses were confirmed by Allen & Wikel (1978) who demonstrated entrapment and/or death of ticks in the exudate. Nevertheless, Tatchell & Moorhouse (1968), Roberts (1968a) did not find significant variation in the engorgement periods for B. microplus, except decline in engorged numbers during secondary exposures despite the varied magnitude of lesions between hosts. Hewetson & Nolan (1968) observed regression in engorged female ticks accompanied by engorged weight drop, increased day of modal drop and reduced egg-hatchability though oviposition was not significantly affected. Gebelhoff (1973) in agreement with Riek (1962) and Allen & Wikel (1978) found only few female ticks completely engorged on calves repeatedly infested and their eggs markedly reduced in number, size and hatchability.

Extreme tissue reaction and behavioural responses could grossly alter factors found in a sedate host. Pavlovsky et al., (1954), cited by Hoogstraal (1956), found hinderance of normal engorgement when large numbers of Hyalomma dromederii fed had caused skin inflammation, a reaction elucidated later by Riek (1962). Similarly, Nuttall (1914B), cited by Hoogstraal, (1956), had described an oedematous swelling consequent to irritation from the long mouthparts of ixodid ticks. The resultant lesion gradually walled off or engulfed the feeding tick. This reaction has been subsequently recorded by Trager (1939a); Riek, (1962); Francis, (1966); Rubaire-Akiiki, (1977); Musatov, (1978).

Moorhouse & Tatchell, (1969), Branagan (1974) and Dr. R. Newson (pers. comm.) observed occasional failure to feed for no apparent reason leading to death unattached or at an early stage of engorgement in R. appendiculatus ticks. Withered ticks which eventually fell off or flaked with epithelial scabs in both susceptible and previously exposed hosts were observed. In addition death in situ of partially engorged female ticks on primary and secondary exposures was found by Riek (1956, 1962), stampa (1959), Hewetson & Nolan (1968) and Bennett (1975). Tatchell (1969b) reported death in argasid ticks which had fed normally on immune rabbits, and Riek (1959) reported failure to moult in other ticks fed similarly. Further observations revealed discoloured

translucent engorged ticks especially on animals in poor condition (Arthur, 1962; Allen, 1973; Allen & Wikel, 1978). Trager (1939a) attributed the phenomenon to host tissue response and tick physiology. O'Kelly & Spiers (1976) reckoned total tick burden was negatively correlated with calf weight gain and total serum protein, albumin and cholestrol, though O'Kelly & Seifert (1970) had stated that this status could break down under larger infestations. Thus other workers, like, Sutherst et al., (1973); Gladney et al., (1973) also showed that nutritional status affected host susceptibility whereby dietary deficiency weakens the resistance.

Trager (1939b) demonstrated acquisition of resistance to single and repeated Dermacentor variabilis infestations. Riek (1962) described an innate and acquired resistance, stating the latter developed from repeated exposures and was primarily associated with a hypersensitivity state. Previously he had associated a heavy mortality during early stages of the life cycle with sensitization. Several workers, such as, Roberts (1968a); Tatchell (1969b); Hewetson (1971); O'Kelly & Spiers (1976), postulated an immunological reaction as a major factor in the control of numbers of ticks maturing on a host and its development during the first parasitic life cycle. Roberts (1968a) suggested that tick rejection might be caused by imbibed host factors, alteration of attachment size creating unsuitable feeding conditions.

Subsequently Bagnall (1978) has demonstrated basophils and their granules in the gut of dead larvae which could have succumbed to lethal effects from these cells. Detachment and reattachment were not a common feature overall in larval instars on resistant animals, though it was found to be common in other studies made by Tatchell & Moorhouse (1968); Kemp & Koudstaal (1971); Bennett (1975) and Allen (1977). Roberts (1968b) demonstrated that 30-100% of the larvae applied failed to mature on individual resistant animals and this was in accord with other studies on host resistance (Trager, 1939b; Snowball, 1956; Moorhouse & Tatchell, 1966; Bagnall & Rothwell, 1974; Bagnall, 1978). Francis (1966) described death in situ associated with the walling off reaction round mouthparts revealed histologically. A common feature to these studies was that the significant rejection occurred during secondary exposures. It was found ^{that} the greatest mortality occurred during the first twenty four hours of infestation irrespective of the instar applied (Bennett, 1975; Roberts 1968b,c). Conversely, where the instar had established on the host the immune response did not significantly influence the feeding of the tick though death of partially and completely fed ticks was observed after successful attachment (Tatchell, 1969a; Branagan, 1974; Kemp, 1978). Hewetson & Lewis (1976) found that fewer ticks matured on cattle infested continuously than on animals

infested intermittently, but much higher numbers applied daily by Roberts (1968c) produced a different response. Riek (1956), Doube & Kemp (1975) recorded death of ticks which was associated with host responses to primary and secondary exposures in different hosts. Stages in the life cycle at which losses may occur resulting from host resistance were investigated. The highest mortality was found in larvae (Allen & Wikel, 1978; Roberts, 1968b; Branagan, 1974), especially in highly resistant animals, though grooming could take an appreciable percentage (Riek, 1962; Bennett, 1969; Koudstaal et al., 1978; Kemp, 1978). However, continuous losses throughout the parasitic cycle were demonstrated also by other workers (Francis, 1966; Kemp et al., 1976; Bennett, 1975).

The inference from studies on tick performance indicated that the net result was a measure of host resistance. Thus the effects of resistance were estimated in terms of numbers successfully fed, engorged weights, duration of life cycle, egg batch size, weight and hatchability (Riek, 1962; Hewetson, 1972). Stampa (1959) and Doube & Kemp (1975) demonstrated delayed or no complete engorgement, premature detachment and death in situ; and they also recorded failure to oviposit and reduced engorged weights. This phenomenon had benefited the infested animals which survived tick paralysis as a result of inhibited tick performance.

Allen & Wikel (1978) demonstrated significantly depressed engorged numbers and weight, and death in situ in Dermacentor andersoni larvae applied to previously exposed guinea pigs. Despite extensive studies, especially in Australia, the mechanism of resistance has not been precisely defined. Further studies undertaken by more workers to characterise the nature of the resistance strongly supported the immunological responses. "Köhler et al., (1967) induced reduced engorged size and reproductive capacity in ticks fed on rabbits repeatedly exposed. They demonstrated humoral antibodies from the rabbits exposed to ticks and their salivary gland extract. Allen (1973) on administration of the immunosuppressant, methotrexate, prevented the acquisition of resistance to D. andersoni larvae in resistant guinea pigs. In contrast, Bagnall & Rothwell (1974) failed to inhibit the immune response against Ixodes holocyclus in guinea pigs treated with promethazine or methotrexate. However, Wikel & Allen (1976b) demonstrated significant blockade of the immune response using cyclophosphamide (immunosuppressant). At the same time they showed the resistance was transferrable by lymph node cells but not by serum from resistant guinea pigs. In corroboration, Kemp (1978) had found no indication that immune plasma or serum caused damage to larvae in vivo or in vitro. The passive administration

of the cells suppressed the engorgement of larvae. The intradermal test in resistant hosts revealed 'delayed skin reactivity'. However, Wikel & Allen (1976a) had found the protection passively induced was not as complete as that afforded by natural exposure. Brossard (1976) demonstrated development of resistance in cattle through repeated exposure to ticks and/or their salivary gland extract and this was associated with humoral antibodies. Kemp (1978) observed that of the multiple effects against larvae, histamine released through antigen-antibody reactions as ticks fed was connected with early detachment of larvae. Such a reaction was associated with accumulation and degranulation of certain leucocytes, e.g. basophils, which have histamine as one of their main constituents. It was thus a common finding that resistant hosts allowed fewer ticks to engorge which were lighter in weight. Bennett (1969) observed declining tick yields from multiple infestations despite harnessing. This was attributed to high challenge facilitated by harnessing leading to an enhanced degree of resistance.

Tatchell & Bennett (1969) cited Zweifach (1965) who considered histamine to be the primary mediator of immunological reactions, thus supporting Riek (1956, 1962) who demonstrated a more vigorous reaction to histamine in the resistant hosts. However, the study by Tatchell & Bennett (1969) indicated that histamine had a secondary role in the mediation of the immunological reactions. Tatchell & Moorhouse (1968) suggested that the pharmacological effect of histamine was advantageous to newly attached larvae.

Conversely, the early detachment of larvae and death of ticks were connected with release of histamine and other pharmacological mediators (Kemp, 1978; Allen, 1973; Koudstaal et al., 1978). Moreover, Tatchell & Bennett (1969) demonstrated increased tick yield on administration of antihistamines.

Histological studies in different hosts have demonstrated distinct immune responses in sensitized hosts (Trager, 1939b; Tatchell & Moorhouse 1968; Allen, 1973, Schleger et al., 1976).

Whilst factors responsible for high mortality remained largely unknown it became apparent that self-grooming was important in reducing the tick survival rates especially in resistant hosts. The gross influence of host behavioural responses on survival of ticks was studied by many workers. Rich (1973) demonstrated that maternal grooming reduced the number of ticks attacking and feeding on calves. This was beneficial as it reduced the incidence of tick paralysis. Similarly Doube & Kemp (1975), working on tick paralysis in cattle, found ticks were lost through grooming. O'Kelly & Spiers (1976) considered that grooming behaviour could be an important component of the resistance mechanism. Thomas (1962) abandoned experiments due to heavy losses in attached ticks as a result of grooming which was not anticipated. He noted that previously exposed animals rubbed against poles and other objects. Kemp (1978)

reported a 20% to 50% loss in ticks through grooming in highly resistant animals. Koudstaal et al., (1978) similarly demonstrated 19% to 54% losses in Boophilus microplus larvae applied to resistant cattle. Snowball (1956) highlighted the influence of self-licking on Boophilus microplus populations by demonstrating a highly significant effect of harnessing in promoting tick survival. The results were corroborated by Riek (1956, 1959, 1962) and Bennett (1969) who demonstrated proportionately higher tick yields in resistant animals exposed to single and multiple infestations. Grooming caused mortality through mechanical means. Snowball (1956) called for recognition of these responses in the study of mechanisms regulating tick populations in the field. However, Hewetson & Nolan (1968) did not consider licking significant. In this regard the predilection site(s) could be a major determinant. Riek (1962) and Roberts (1968a) noted that compulsive licking and rubbing occurred in some, but not all animals which were highly resistant to ticks. No specific correlation was made in these studies between the intensity of grooming and degree of resistance, though it was commonly observed that highly resistant animals groomed most vigorously. However, Roberts (1968a) found that pruritis, which leads to grooming, was associated with higher resistance but noted that many highly resistant animals did not evince this severe reaction

to infestation. Thus Riek (1962) found that pruritis was most marked in highly resistant cattle which developed raw denuded areas by vigorous licking. Hewetson & Nolan (1968) demonstrated the same reaction associated with the appearance of papules and exudation in previously infested cattle.

1.2.3. CLINICAL AND PATHOLOGICAL MANIFESTATIONS

These are mediated through the direct activity of tick feeding and the interaction between tick secretions and host tissues. Certain parameters show distinct differences between primary and secondary tick exposures though an overlap occurs in others where the distinction can only be judged by the intensity and onset of the reactions (Tatchell & Moorhouse, 1968). It is noteworthy that most of the reviewed studies involved Boophilus microplus, a one host tick, in Australia, whereas relatively little has been done on Rhipicephalus appendiculatus in Africa. However, Neitz (1962) assumed that light rhipicephaline infestations did not cause any clinical toxic reaction. Tatchell & Binnington (1973) postulated a pharmacological activity in tick saliva. During the assay of an active constituent of B. microplus oral secretion they induced an increased capillary permeability after intradermal injection of tick saliva into the bovine host. The response was reckoned to facilitate tick feeding. Moorhouse & Tatchell (1969) had found the

same response in the superficial skin capillaries soon after attachment of larval ticks which was suggestive of a pharmacologically active material in the infected saliva. Neitz et al., (1969) demonstrated a lethal factor in saliva of Ornithodoros savignyi associated with enzymatic activities. Besides death, other systemic disturbances e.g. body temperature derangement, were also induced. Theiler (1921) induced extreme pallor culminating in death in a horse and a heifer and the effects were attributed to exsanguination from massive infestation. Tatchell & Moorhouse (1968) working with ^a/B. microplus-bovine system concluded that the feeding lesion of the tick was the result of an interaction between the tick and its host. They contended that it was neither the result of single mechanical trauma, nor of lysis of the host tissues by salivary secretions alone, nor was it solely a result of an inflammatory response. Berenberg et al., (1972) demonstrated the neutrophil-dependent nature of the tissue damage and the results emphasised that the host's cellular reaction to the attached and feeding tick appeared to be crucial to the subsequent development of tissue damage. Tatchell & Moorhouse (1970) had shown that tissue damage at the site of a tick bite was neutrophil dependent. Gregson (1970) found that the tick cement laid in the host tissue by Dermacentor sp. was antigenic though Tatchell & Moorhouse (1966) concluded that the cement from B. microplus

was inactive. Riek (1962), working with previously exposed animals, indicated that immunological changes can affect the susceptibility of cattle to infestation. Thus, it was necessary to consider the possibility of immunological phenomena when selecting experimental hosts for ^{the} study of feeding processes of the tick. This remark supported the histological findings made by Trager (1939b) who showed differences in the reactions of resistant and susceptible guinea pigs to Dermacentor variabilis. Similarly, Allen & Wikel (1978) found significant clinical and histological differences between resistant and susceptible guinea pigs to Dermacentor andersoni larvae. Resistant hosts developed vesiculation and gross thickening of the ear and ^amarked basophilia in ^{the}dermis and epidermis and in the peripheral circulation. Wagland (1975) and Hewetson (1971) demonstrated marked differences in cardiovascular and cutaneous responses between primary and secondary exposures to Boophilus microplus. The responses to secondary exposures were more severe and were associated with immunological reactions e.g. urticaria, pruritis. On the other hand, van Rensburg (1959) observed ^{that} clinical signs were severe in susceptible and mild in immune cattle exposed to Rhipicephalus spp..

Dermatoses of localised or generalised nature have been identified with different genera of ticks, and the

observed signs included: erythema, cellulitis and swelling and haemorrhage around bite sites, necrosis, ulceration, exudative eczema, otoacariasis complicated by Corynebacterium pyogenes infection and sclerotisation in persistently infested areas. Thomas & Neitz (1958) and van Rensburg (1959) while investigating rhipicephaline toxicosis in cattle observed intense congestion (erythema) at the bite-sites on ears and eyelids; swelling and oedema especially round^{the}/head, eyelids and ears; discrete or confluent papules at attachment sites; blood stained lymph tapped from swellings; exudation that "scalded" the skin, abscessation of^{the}/external ear (otoacariasis); and pinna distortion sometimes accompanying recovery. Riek (1962) observed, especially in resistant hosts, papular swelling (urticarial) at bite sites, extensive serous exudation which formed thick encrusts, alopecia in encrusted areas, oedema in infested areas, blisters turning pustular and finally leaving pitted scars. Similar responses dominated by papules, oedema, exudation and pitted-scars were demonstrated by Hewetson & Nolan (1968) and Hewetson (1971). Doube & Kemp (1975), studying tick paralysis in calves, found little trauma and some erythema around bite sites during primary exposure. Hewetson (1971) initially found no significant reaction at bite sites during primary exposure to Boophilus microplus but later in the life cycle

papules and oedema developed. However, Doube & Kemp (1975) found that calves exposed previously reacted more strongly and developed urticarial swellings, serous exudate and hard encrustation around bite sites and ulcers under scabs. Arthur (1962) reported similar inflammatory reactions, however, the associated secondary complications produced serious consequences, e.g. canker of ear connected with Dermatophilus sp. infection, myiasis with serious sequelae in the affected ear. Repeated exposures provoked defensive skin reactions, thus, Köhler et al., (1967) demonstrated the "sclerotisation" on/ears and back of rabbits infested with Hyalomma anatolicum excavatum and Rhipicephalus sanguineus. Göksu and Özgencil (1970) studied lesions the caused by ticks on/external ears in sheep and found that Rhipicephalus sanguineus induced a reaction of hyperkeratotic nature. Tatchell & Moorhouse (1968), in contrast, did not find epidermal proliferation and hyperkeratization in their studies on Boophilus microplus. Gaafar (1972) describes keratinization as a defensive reaction against excessive provocation to the epidermal basal cell layer. This is a non-specific reaction to skin irritants. Multiple exposures frequently induced marked pruritis, also recorded in the above studies, that exacerbated the dermatosis reactions.

Secondary exposures were thus associated with new

or more serious responses relative to responses induced by primary exposures. The responses commonly recorded were pruritis, oedema, exudation, papules (urticarial) and bleb formation, which occurred in different combinations. Pruritis elicited in 'immune' (repetitively challenged by ticks) animals caused much discomfort and vigorous grooming which led to trauma-^{and}tisation/^{was} also recorded by Theiler (1921), Clifford (1954), Hewetson & Nolan (1968), Hewetson (1971, 1972). However, in man the irritation was induced during primary exposure (Oxer & Ricardo, 1942; van Rensburg; 1959; Marshall, 1966). Intense oedema associated with other inflammatory reactions, especially round attachment areas, was observed in laboratory and field cases (Tatchell, 1969a, Thomas & Neitz, 1958; Hewetson, 1971; Branagan 1974). Continued bleeding from detachment and traumatization wounds occurred (Macleod, 1933; Thomas & Neitz 1958). Varied degree of serous or serofibrinous exudation from bite wounds or broken blebs formed encrustations which "scalded" the underlying skin, causing erosions, loss of hair or ulcers (Thomas & Neitz, 1958; Riek, 1962). Bleb formation and papules were prominent features in previously exposed hosts but their development was only reported by Trager (1939a), Riek, (1954^b, 1956, 1962), Branagan (1974), Hewetson, (1971), Schleger et al., (1976), Allen & Wikel, (1978). Histological investigations on hosts

with such exudation commonly revealed massive infiltration of eosinophils and/or basophils, showing marked degranulation which might have a major role in the development of dropsical changes (Allen, 1973; Moorhouse & Tatchell, 1966; 1969; Allen & Wikel, 1978; Burghardt et al., 1951).

Healing of integumentary lesions was generally rapid and occasionally left 'pitted' scars (Riek, 1962; Tatchell & Moorhouse, 1968; Doube & Kemp, 1975). However, severe and/or persistent reactions caused secondary infections and morphological transformation or necrosis and sloughing. The secondary infections involved abscessation locally and in drainage lymph nodes, pyaemia (Hoogstraal, 1956; van Rensburg, 1959; Macleod, 1933; Thomas & Neitz, 1958; Wood, 1968), myiasis (Arthur, 1962; Seebeck et al., 1971), streptothricosis (Arthur, 1962), ^{and} mastitis (Clifford, 1954; du Toit & Theiler, 1964; Bonsma, 1944). Distortion or loss of organs, e.g. ears, tail tip, were reported by Clifford (1954) and du Toit & Theiler (1964). The ear distortion could be a sequel to necrotising effects of the tick secretion and of scarring (Arthur, 1962; Whitwell, 1978). Alopecia (depilation), an important differential feature in dermatoses, is reported, developing at bite sites and adjacent skin encrusted with exudate or traumatised through grooming (Philip et al., 1935; Riek, 1962; Marshall, 1966; Gebelhoff, 1973). Wilkinson (1955) reported extensive loss of hair in

heavily infested animals.

A considerable wealth of physiopathological data on skin responses has been recorded but there is proportionately little on the reactions in other systems. However, the interpretation of the responses in these systems is complicated by concurrent infections and nutritional deficiencies. Thus other parameters investigated included lymphatics, cardiovascular function, blood composition, respiratory patterns, function of digestive and urinary systems, and the nervous responses (pruritis). It is worth noting that in many cases the recorded physiopathological reactions were not specifically planned in the studies reviewed and therefore were secondary findings. Lymphangitis and lymphadenitis of marked degree were detected in the infested regions by many workers (Feder, 1944; Dr. Taylor, cited by Hoogstraal, 1956; Thomas & Neitz, 1958; Hewetson, 1971). Gaafar (1972) states that enlargement and induration of regional lymph nodes are usually observed following arthropod bites. However, van Rensburg (1959) noted a lack of lymphadenopathy in the nodes during tick feeding in chronically infested animals. Lymph node abscesses were frequently found at slaughter though spontaneous rupture of the abscesses occurred in antemortem cases.

Temperature rise associated with tick engorgement in man and animals was reported by Feder (1944); Riek (1956); Neitz (1962). van Rensburg (1959) reported frequent pyrexia and apathy in calves massively infested with B. decoloratus. Conversely, Jellison & Kohls (1938) and Riek (1956) recorded subnormal temperatures for one to two days prior to death. Wagland (1975) induced mono- and diphasic temperature patterns on primary and secondary tick exposures respectively. The secondary phase was associated with the allergic reaction in sensitized hosts. Budumyan (1978) recorded elevations in temperature, pulse and respiration (TPR) in sheep with tick toxicosis. Inoculation of tick salivary secretion and tissue extracts induced temperature derangement (Neitz et al., 1969; Riek, 1957b).

There is limited observation on the visible mucous membranes and their secretions. However, the available records show only pallor (caused by anaemia) (Philip et al., 1935), conjunctival congestion and lachrymation (Thomas & Neitz, 1958) and nasal catarrhal secretion (Philip et al., 1935, Arthur, 1962). Arthur (1962) further reported discharges springing from nostrils, eyes and throat as a result of serious otomyiasis.

Concurrent with temperature, pulse and respiratory disturbance, changes in body condition, physique and demeanour were recorded by many of the reviewed workers. Alteration in demeanour revealed depression, listlessness, restlessness, hyperaesthesia and pain (Philip et al., 1935;

Arthur, 1962; Thomas & Neitz, 1958). Deterioration in body condition, growth rate, general physique leading to prostration and death were reported also by Macleod (1933), Clifford (1954), Francis (1960), Little (1963) and Seebeck et al., (1971).

Anorexia, ruminal atony and increased urination were found in infested animals by several workers (Neitz, 1962; Riek, 1956) but abdominal pain and vomiting also occurred in man (Feder, 1944).

A number of reports carry limited data and/or general observations without supporting figures and are further complicated by intercurrent problems, e.g. vector-borne or secondary bacterial infections. Broadly the changes were related to exsanguination, haemorrhage and hypoplasia in the haemopoietic system. Critical and fatal blood loss under heavy infestation were observed in cattle and horse (Theiler, 1921), sheep and rabbits (Philip et al., 1935) and rabbits (Jellison & Kohls, 1938). Derangement in red blood cell (RBC) indices, sometimes critical, generally correlated with infestation densities under heavy challenge (Riek, 1957a; van Rensburg, 1959; Johnston & Haydock, 1969; O'Kelly et al., 1971; Gladney et al., 1973; Williams et al., 1978). Development of reticulocytosis and other regenerative features reported by workers such as, Little (1963), O'Kelly & Seifert (1969), O'Kelly et al., (1971), Gebelhoff (1973), discounted the aplastic anaemia response suggested by Thomas & Neitz (1958).

Significant host responses to high tick infestations were generally associated with decline in white blood cell counts (Williams, 1978; Williams et al., 1977; van Rensburg, 1959). Resistant hosts showed eosinophilia (van Rensburg / O'Kelly et al., 1971; Hewetson, 1971), though some workers did not find it (Riek, 1957a; van Rensburg, 1959; Williams et al., 1978). Other differential count changes which were not always consistent included, lymphocytosis (van Rensburg, 1959; O'Kelly et al., 1971) or lymphopenia (Williams, 1978; Williams et al., 1978), neutrophilia (Williams et al., 1978), or neutropenia (van Rensburg 1959; O'Kelly et al., 1971), and basophilia (Williams 1978; Williams et al., 1978; Allen & Wikel, 1978).

Generally serum total protein (T.P.) did not show a significant decline on account of the inverse relationship between albumin and globulin levels. van Rensburg (1959) confirmed this observation during the initial period of infestation, but subsequently found the appearance of cachexia was typified by unusually low total protein levels. Despite the fall in total protein, the globulin levels remained persistently high. Cachexia was apparently characteristic of the toxicosis syndrome. O'Kelly et al., (1971) similarly found that the specific effect of ticks depressed the serum protein and elevated the globulin concentration. Thus O'Kelly & Seifert (1970) had stated that serum total protein was an unreliable measure of tick effects and recommended serum albumin as

a more sensitive index. However, many workers seem not to have identified this index for study of specific tick effects. Riek (1957a) noted a decrease and then a return to normal in total serum protein. O'Kelly & Spiers (1976) found that ^{the}total tick load carried correlated negatively with calf weight gain, concentration of serum albumin and total protein. Williams et al., (1978) registered increases in total serum protein (TSP), serum globulins and plasma fibrinogen and decreases in the albumin/globulin (A/G) ratio.

Riek (1955, 1962) demonstrated significantly elevated blood histamine levels in resistant hosts when reinfested. Many other workers have also found that challenge of sensitized hosts produced elevated blood levels (Code, 1939) or tissue reactions associated with release of histamine and related compounds (Kemp, 1978; Moorhouse & Tatchell, 1966, 1969; Tatchell & Bennett 1969; Allen & Wikel, 1978; Allen, 1973). Other biochemical parameters showing consistent changes were cholesterol, and phospholipids. O'Kelly et al., (1971) demonstrated that the total tick numbers carried correlated negatively with albumin and free cholesterol concentration, lactic dehydrogenase (LDH), serum glutamic oxalacetate transaminase (SGOT) and monocyte ^{numbers}/ and positively with serum globulin concentration. Another, rarely mentioned, metabolite is the serum glucose which remained unaltered in the studies made by O'Kelly et al., (1971)

the
on/blood composition of cattle infested with Boophilus
microplus.

Many reports of death attributable to specific and contributory effects of different tick species were made, though a large proportion were not proven. The specific effects could be mediated through exsanguination (Theiler, 1921; Jellison & Kohls, 1938; Johnston & Haydock, 1969) and toxicosis (Neitz et al., 1969; Thomas & Neitz, 1958; Neitz, 1962). Exsanguination, thought to cause sudden death in large animals infested with Ornithodoros savignyi, is reported by Hoogstraal (1956) and du Toit & Theiler (1964). Such sudden death in large animals was shown by Howell et al., (1975) to be caused by the toxic salivary secretion as the principal factor. The toxicity was associated with proteolytic and cholinesterase activities. Neitz (1962) and Dr. M.P. Cunningham (personal communication) observed very early symptoms followed by death in susceptible cattle carrying massive Rhipicephalus appendiculatus infestations. Several workers believed that the contributory effects operated in consort of infection and/or "deprivation" (Johnston & Haydock, 1969; Philip et al., 1935; Clifford, 1954; Thomas & Neitz, 1958).

Comprehensive reports on autopsy findings are scarce and where ticks effects were contributory the interpretation of such results has been difficult. In general cardiovascular and haemopoietic changes were more frequent. Thus the main features reported were:

pallor and watery blood and oedematous changes (Theiler, 1921; Philip et al., 1935; Jellison & Kohls, 1938; Thomas & Neitz, 1958; Lutu, unpublished), haemorrhages mostly in attachment areas (Philip et al., 1935, Jellinson & Kohls, 1938; Thomas & Neitz; 1958), fatty degeneration of liver parenchyma surrounding the intralobular vein (Neitz, 1962). In addition, Thomas & Neitz (1958) reported severe wasting in tissues, e.g. muscle, spleen, fat; enlargement, degeneration and abscessation in regional lymph nodes; and cutaneous thickening. van Rensburg (1959) further noted a relationship between the size and structure of adrenal glands and severity of skin reactions to infestation. Riek (1957b) inoculated tick tissue extracts which induced congestion, oedema and necrosis locally, congestion and fatty liver changes, congestion and blood vessel engorgement in the kidneys. However, use of such extracts, instead of salivary secretion, introduces complications from other active principles which can act alone or synergistically to precipitate toxic effects. This concurs with Gaafar (1972) who discusses the marked variability in the composition of arthropod somatic tissue. By analogy, Willadsen et al., (1978) thus state, ...'any investigation of the immunological basis of resistance to ticks will be complicated if the only test material available is a crude tick extract containing a mixture of antigens'. On the other hand, Neitz (1962) reported that certain animals, which succumbed to massive

Dermaacentor albipictus infestations, showed little obvious change in the organs on autopsy.

1.2.4. RESISTANCE TO TICK INFESTATION

Successive tick infestations of different densities in cattle and other hosts do induce physiopathological responses summed up as manifestations of resistance. Many workers, especially in Australia, have investigated the development of this phenomenon and its application to biological control of the parasite population. Neitz (1962) noted that immunity to Rhipicephalus appendiculatus was maintained by periodic infestations. The accumulated evidence indicates that a major component of the resistance is acquired (Trager, 1939b; Riek, 1962; Roberts, 1968a; Tatchell & Bennett, 1969; Roberts & Kerr, 1976; Allen & Wikel, 1978; Willadsen et al., 1978; Bagnall, 1978). The acquired resistance may last for an indefinite period depending on the degree of individual susceptibility (Riek, 1956). Its assessment was based on tick performance, (Wilkinson, 1962; Riek, 1956, 1962; Wharton et al., 1970b; Wagland 1978), clinical hypersensitivity (Branagan, 1974; Riek, 1954b, 1956, 1962; Willadsen et al., 1978), histological reactions (Trager, 1939a; Tatchell & Moorhouse, 1968; Schleger et al., 1976), development of humoral antibodies and cell-mediated immunity (Wikel & Allen, 1978; Willadsen et al., 1978; Brossard, 1976 and Trager, 1939b).

However, the mechanism through which the tick is affected is not understood, though effects of histamine and its analogues demonstrated in resistant hosts have been associated with tick mortality (Kemp, 1978; Tatchell & Bennett, 1969; Riek, 1962, Bagnall & Rothwell, 1974). Inflammatory reactions were also connected with damage to the parasite (Allen, 1973; Trager, 1939a; Tatchell & Moorhouse, 1968; Doube & Kemp, 1975). Bagnall (1978) associated larval death with ingestion of basophils and their granules. The host responses were closely associated with eosinophil and basophil infiltrations but these cells were less frequently found in ^{the} peripheral circulation. These infiltrations accompanied clinical hypersensitivity reactions. However, defensive cutaneous reactions, e.g. walling off, affecting tick feeding, have been reported in both susceptible and sensitized hosts (Tatchell & Moorhouse, 1970; Trager, 1939a; Francis, 1966; Rubaire-Akiiki 1977; Riek, 1959 ; Berenberg et al., 1972). The net effect of these extensive skin studies has been suppression of tick performance, i.e. attachment, engorgement numbers and weights and general viability.

The more subtle effects upon tick performance resulting from repeated exposures have been associated with cellular and humoral immune components (Trager, 1939b; Riek, 1962; Wikel and Allen, 1976a, 1978; Bagnall and Doube, 1975). However, in some cases humoral antibodies passively transferred did not produce significant

effects upon tick feeding (Irvin et al., 1973; Bagnall & Rothwell, 1974; Kemp, 1978; Bagnall, 1978). Contrarily the passive administration of lymphoid cell preparations from "immune" hosts affected the feeding of ticks (Bagnall, 1978; Wikel & Allen, 1978).

The hypersensitivity state has been induced artificially by inoculation of tick saliva or tissue extracts and naturally through tick feeding. The resultant sensitization has been recognised by the visible clinical and histopathological responses in different hosts. Histologically major responses associated with tissue basophilia and eosinophilia and secondary vascular changes with resultant degenerative changes are recorded by many workers, such as, Cowdry & Danks (1933), Rubaire-Akiiki (1977), Riek (1962), Schleger et al., (1976), Allen & Wikel (1978). Bagnall (1978) observed an apparent correlation between tissue basophilia and level of immunity noticed. These changes may progress into clinically discernible signs, viz: oedematous swellings (Thomas & Neitz, 1958; Hewetson, 1971, 1972; Branagan, 1974); bleb formation (Riek, 1962; Allen & Wikel, 1978); excessive exudation accompanied by alopecia (Thomas & Neitz, 1958; Riek, 1962; Branagan, 1974; Hewetson, 1971, 1972); urticaria (Riek, 1962; Hewetson, 1971); ulcer and pit-scars under crusts around the hypostome (Hewetson 1971; Riek, 1962). Thus these changes prevented engorgement or led to death in situ and shedding of ticks along with necrotic tissue (Branagan, 1974; Riek, 1962).

Except when extreme reactions led to secondary complications these immune responses indirectly saved animals from specific tick transmitted infections (Doubé & Kemp, 1975). The pruritis associated with release of pharmacological mediators of immune responses, e.g. histamine, caused grooming that threatened tick survival (Riek, 1962; Tatchell & Bennett, 1969; Koudstaal et al., 1978; Kemp, 1978).

The existence of the hypersensitivity state has been tested by feeding ticks or by the intradermal inoculation of tick saliva secretion or tissue extracts. The tested hosts were either susceptible, passively sensitized with serum and immune cells, i.e. lymph nodes or lymphocytes (Wikel & Allen, 1976a, 1978; Trager, 1939a; Bagnall, 1978; Riek, 1962), or actively sensitized by exposure to ticks or their products (Riek, 1954b; 1958; Geczy et al., 1971, Gregson, 1970). Riek (1962) on testing^{the}/resistance state in cattle found no direct correlation between the degree of intradermal test reaction and intensity of the hypersensitivity state. Thus he found no evidence of skin hypersensitivity even after six spaced exposures and yet one animal reacted only 17 days after initial infestation. However, Willadsen et al., (1978) found significant correlations between resistance and the immediate hypersensitivity reactions.

Further studies on the induced immunological responses have revealed a number of in vitro tests for antibodies, e.g. precipitating, complement fixation, haemagglutination,

fluorescent (Trager, 1939b; Köhler et al., 1967; Brossard, 1976; Bagnall, 1978). Brossard (1976) claimed that there was a positive relationship between antibody titres, determined by indirect immunofluorescence and immunoelectrophoresis, and the development of resistance. Willadsen et al., (1978) disputed this claim and in their study demonstrated a significant correlation between the resistance and agglutinating antibody levels. On the other hand Riek (1962) detected no relationship between humoral antibody levels and degree of resistance. Though a wide range of antibodies has been explored the relevance to tick population remains uncertain. Use of immunosuppressants has demonstrated the basis of immunological responses in the manifestation of resistance to tick feeding (Allen, 1973, Allen & Wikel, 1978). However, Bagnall & Rothwell (1974) failed to inhibit the response to Ixodes holocyclus in guinea pigs treated with promethazine and methotrexate.

In contrast, the susceptible hosts exhibit very little or none of the responses on challenge with ticks and their products (Koudstaal et al., 1978; Hewetson, 1971). However, evidence of resistance was detected in less than two weeks of primary tick exposure (Roberts, 1968a; Allen, 1973; Irvin et al., 1973).

1.3.

CONCLUSIONS

A wide range of responses associated with tick infestation were reported in different hosts. Several genera of ticks and hosts were studied but the Boophilus microplus - cattle system was the most extensively investigated in Australia. The responses initially attributed to tick effects were predominantly encountered during investigations of tick-borne infections and therefore were incidental. However, many workers tried to distinguish such signs that were inconsistent with the transmissible infections but it proved difficult because of the concurrent interaction from tick-borne diseases. Nonetheless, conclusions and conjectures were drawn asserting the significance of such findings. The health disturbances attributed to tick effects included unthriftiness, lowered productivity, exhaustion and death, toxicoses, aggravation of disease syndromes, predisposition to secondary infections, and increased susceptibility to tick-borne diseases through suppression of reticuloendothelial system (RES) defensive mechanism. On the other hand further critical studies in non-infected hosts were carried out to confirm the observed features or to demonstrate the speculated phenomena.

Thus the incidental findings associated with tick effects were as follows.

- i) Physical signs. These were manifested in various systems. The cardiovascular system showed critical or fatal anaemia, and unremitting fever (pyrexia). Circulatory changes in the skin attributed to tick attachment were erythema, oedema or prolonged bleeding from bite wounds. Other cutaneous reactions were nodules (papules), exudation, bleb formation, depilation and hyperkeratosis. Irritation provoked grooming which caused skin abrasions. Regional lymph nodes were enlarged. The sequelae to infestation were suppurative wounds, pyaemia, lymph node abscessation, myiasis, deformities and death. Death, especially when sudden, was presumed to result from exsanguination or intoxication during massive infestation. The surviving animals were unthrifty and showed long convalescence.
- ii) Haematological findings. These were of limited precision and revealed a decline in red blood cell and total protein picture.
- iii) Autopsies. Though few were reported they revealed prominent changes in circulatory, lymphatic and muscular systems. The circulatory changes included congestion, haemorrhages, oedema or pallor. The spleen and muscles were wasted.
- iv) Existence of tick-free cattle. This occurred with some cattle showing thick encrustations on the skin in undipped herds constantly challenged by ticks. Some animals carried fewer and poorly fed ticks during repeated exposures.

v) Paralysis and dermatosis. The specific syndromes were attributed to tick toxicosis. However, the toxicoses are not specifically reviewed in the present study.

The major features explored in the further critical studies were as follows.

i) Intoxication, reflected in sudden death, debility and low productivity involving growth and weight gains.

ii) Resistance in relation to tick performance, hypersensitivity state, other immunological responses tested in vivo and in vitro, and biochemical responses such as histamine levels.

iii) Extensive investigations on the gross and histological skin reactions. The findings revealed the effects of mechanical injury, degradation caused by enzymatic activity, pharmacologically and immunologically mediated responses.

The resistance phenomenon stimulated tremendous interest and is considered an important biological factor in the regulation of tick populations. Thus extensive past and current studies have been undertaken in both cattle and laboratory animals. Because of the economic importance of B. microplus the Australian workers are intensively studying the heritability of resistance in cattle. However, this is a long term research as the essential genetic characteristics are difficult to identify. Despite the vast amount of work done the

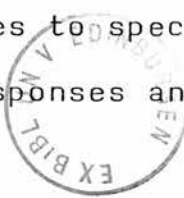
mechanism of the resistance phenomenon remains undefined.

In contrast studies on the clinico-pathological changes in other systems besides the skin have had comparatively little attention in cattle. The recorded investigations covered effects on the circulatory system, feed intake and convertibility and growth estimated in weight gains. Interpretation of the findings was complicated by ^{the} multiplicity of factors involved in the experimental design, and consequently some responses were designated direct and others indirect. Full autopsy reports are scarce and comprehensive systematic studies are lacking, which could be attributed to the precedence given to transmissible diseases and economic considerations. The economic constraints apply to large cattle numbers, space and feeding and processing of material.

The methods adopted in these studies and observations varied from natural exposure through conventional farm practices to animals being experimentally confined in infested paddocks or manually infested in stalls or inoculated with tick tissue extracts. Different combinations of these methods were also used. The animals used were non-infected or premune to tick-borne diseases at the start of the trials. The premune state was deliberate or unplanned depending on the source of animals and design of the experiments. Pre-munisation aimed at arresting uncontrolled infections

(heartwater, babesiosis and anaplasmosis) from vector ticks to which the cattle were exposed in the studies. However, some of the premune animals developed frank infections under massive tick infestation. Furthermore use of infected ticks caused overt disease in animals which were previously healthy. Nonetheless, fewer studies were carried out on non-infected tick-cattle systems. The density of successful tick infestations varied, especially during repeated exposures, despite standardisation of the initial numbers applied artificially. This influenced the severity of the responses. Moreover, variation in host susceptibility to different and even the same kind of tick species was commonly demonstrated. Therefore derivation of concrete conclusions required large cattle numbers and non-infected tick-host systems. Thus the limitations of studies on the clinical-pathological responses to tick effects, especially related to the Rhipicephalus appendiculatus - cattle system, can be summed up as follows.

- 1) Concurrent tick-borne infections and premune status which was not protective.
- ii) Initial findings being fragmentary, limited in accuracy and incidental.
- iii) Few comprehensive systematic studies and some of which investigated only a limited number of parameters. Scarcity of autopsy reports.
- iv) Identified responses to specific tick effects confounded by indirect responses and secondary infections.



- v) Difficulty in interpreting and relating information derived from different tick-host systems. This was compounded by varied host susceptibility and extrinsic factors, like quality of nutrition.
- vi) Relegation of such studies to secondary place relative to more important tick-borne diseases.
- vii) Availability of funds for undertaking these specific studies.

Rhipicephalus appendiculatus remains a very important tick in Africa where it transmits several serious pathogens and can cause severe direct effects. Cattle infected naturally or experimentally with tick-borne pathogens present physiopathological signs which are inconsistent with the known disease syndromes and such signs could be attributed to tick effects. Therefore further critical study on the clinical-pathological responses to R. appendiculatus effects themselves in non-infected bovine host is indicated. Thus the present study aimed at an investigation of these clinical-pathological responses in cattle (calves) infested repeatedly with Rhipicephalus appendiculatus.

CHAPTER 2

MATERIALS AND METHODS

2.1. This study was carried out at Nairobi University, Faculty of Veterinary Medicine in the department of Clinical Studies, Kabete, Kenya. The clinical-pathological features investigated, described in more detail later, included physical signs, haematology and blood biochemistry and gross autopsy changes. The scope of the parameters recorded for laboratory estimations was predominantly determined by facilities for handling and processing material. However, the biochemistry was limited to three parameters, viz. serum protein (S.P.), immunoglobulins (I.G.) and serum glucose (S.G.), meant to illustrate the direct effect of tick feeding, antigenic stimulus of tick saliva on the reticuloendothelial system (RES) and disturbance in body metabolism. The parameters recorded for physical (clinical) signs were based on a clinical examination format used in our clinics.

Bos taurus breeds were chosen because of their higher susceptibility to tick effects than the Bos indicus (Zebus) (Riek, 1962; Wilkinson, 1955; Utech et al., 1978b). A few jersey animals were included though the breed develops tick resistance comparable to Zebus (Francis, 1966). However, they were used because of the limited supply of suitable animals. Calves, as opposed to adult cattle, were more expedient to work with

because of cost and handling facilities. They were obtained from farms known to be free of tick-borne diseases for years and where vigorous tick cleansing was carried out. Before experiments they were kept free of ticks by twice weekly spraying with organo-phosphorus (Delnav-) or toxaphene ixodicides and confined to isolated stalls. A high nutrition ration was fed during rearing and experimentation.

Non-infected adult Rhipicephalus appendiculatus stock for initial trials was obtained from a long established colony reared mainly on rabbits at the former East African Veterinary Research Organisation (EAVRO), Muguga, Kenya, now known as the Veterinary Division, Kenya Agricultural Research Institute. Ticks for subsequent trials were bred from the initial stock supplied and reared on calves. All three developmental stages (instars) were applied individually and concurrently to separate groups of animals. The ticks were applied to the ears predominantly and other parts of body facilitated by calico sleeves and tranquillizers respectively. During secondary experiments guinea pigs were infested similarly but the sleeve was fitted around the trunk. Each instar was allowed to feed to complete engorgement. The density, number and frequency of infestation varied. Thus a maximum of ten successive exposures at seven to fourteen day intervals were planned for each host under the different regimes.

Daily physical (clinical) examinations during exposures were carried out and from time to time all calves were screened for tick-borne disease by blood and lymph node smears. Animals that developed severe secondary infections, such as lymph node abscessation, were withdrawn and slaughtered. Laboratory samples were collected before exposure and thereafter once during each infestation. Tick performance was observed for rate of attachment, feeding to engorgement, viability during and after feeding, engorged numbers and weights, moulting rates and reproductive capacity. Autopsies were performed on dead calves and guinea pigs and ^{on} a few calves that were slaughtered because of secondary complications or at end of the experiments.

The diverse clinical-pathological responses induced were further subjected to secondary experiments which were based on four hypotheses. The hypotheses and the methods for their investigation are stated in detail under section 2.16. The procedures followed were the same as for the primary experiments. However, investigations on haematology, biochemistry and tick performance were limited. Both calves and guinea pigs served as hosts. Further excitants, salivary gland extract (SGE) from female Rhipicephalus appendiculatus ticks and immune serum, were introduced. All three instars were applied individually to calves and guinea pigs and fed to complete engorgement. Ticks and SGE were

used for testing the hypersensitivity state in the sensitized hosts. Antibiotics administered prophylactically and antihistamines for demonstrating their action on^{the} responses to tick effects were utilised. Thus the four secondary experiments were designed to test the effects of the tick salivary secretion agent(s), henceforth referred to as the agent(s). The effects tested were as follows, stated as null hypotheses.

(a) The agent(s) cannot provoke clinical-pathological responses on primary exposure; b) the agent(s) cannot produce a clinical-pathological effect that is passively transmissible by immune serum; c) the agent(s) cannot produce a local reaction of a type that can be blocked by antihistamines, and d) there is no difference between the effects induced by primary and repeated exposures to the agent(s). Similarly autopsies were performed on dead animals and the gross pathological changes recorded.

ANIMALS, TICKS, DRUGS, REAGENTS, EQUIPMENT AND PROCEDURES

2.2. EXPERIMENTAL HOSTS

2.2.1. Calves. Purebred Bos taurus and their crosses comprising ayrshire, friesland, guernsey and jersey breeds and predominantly males, were used in this study. Calves 1 to 8 weeks old were obtained from farms free of tick-borne disease, where tick control was vigorously

practised. They were then reared in a tick-free environment. The regimes for tick control on these farms were based on ^{the} use of ixodocides, fencing and pasture management. Isolated stalls were also used at the premises where the experiments were carried out.

Cows were regularly inspected at milking and other stock checked on changing pasture and coming to the dip or spray race for visible fed ticks. The dip-testing service offered by Wellcome (Kenya) was regularly used by big farms to ensure that the dip(s) worked efficiently. On smaller farms inspection was similarly followed and animals were hand sprayed using freshly made spray fluid. Dipping or spraying was normally done once a week depending on the residual effect of the ixodocide, absence of fed ticks, state of fencing and season. The authorised ixodocides were the organophosphorus (Delnav, Cooper), Carbamates (Sevin) (Wellcome) and toxaphene compounds. Where the situation posed problems cleansing was done twice a week. However, an improvement on the dipping or spraying, especially on small herds, was the use of ixodocides in an oil base marketed as 'tick grease' dressing. The greases commonly contained gamma-isomer benzene hexachloride and were used between dipping or spraying. Specific sites, such as ears, base of tail, axillae, dewclaws, were dressed. Fencing, using five wire strands which had to be well maintained, was another

measure employed to prevent tick introduction from infested areas by cattle from within or outside the farm. On farms crossed by highways double fences with cleared track between them were erected at the boundaries. Though these erected fences were well maintained dogs and small game still crossed freely. Thus the compromise was strict surveillance for fed ticks and tick-borne disease. On the particular farm where some of the calves were reared the fences for the calf paddocks were reinforced with chicken wire to arrest movement of dogs and small game. The pasture management practice involved ploughing up fields and sowing them with grain for a number of seasons during which all adult ticks will die of starvation or dehydration. On the premises for the experiments the stalls were often cleaned out by steam and the front yard exposed to the sun was hosed and scrubbed daily. A moat filled with water surrounded the outside of the stalls. Control calves and those not being used were hand sprayed once or twice a week using toxaphene or organophosphorus sprays. Sprayed calves were kept in separate stalls away from the experimentally infested ones to prevent ixodicide contamination which could inhibit the survival of the ticks. Besides, all calves were thoroughly washed before being experimentally infested.

All calves received colostrum and were subsequently fed milk and milk substitutes before weaning on to a

predominantly grass and lucerne hay and wheat bran ration supplemented with young stock pencils. Minerals were always provided. Initially I reared the calves entirely in-doors, but this was modified owing to losses from nutritional scours and respiratory infections in the younger calves. Newly acquired calves were then reared on a tick free farm where they could graze for part of the day. They were put into stalls later for the experiments. Until the calves were transferred to the stalls spraying was done once or twice a week and supplemented with tick grease dressing. Routine anthelmintic and coccidiostat treatments were given and the calves were screened for active tick-borne diseases using blood and lymph node preparations. Tetramisole hydrochloride drench 1.5% (w/v) or injection 7.5% (w/v) (Nilverm - I.C.I.) against helminths and sulphadimidine sodium tablets or amprolium hydrochloride 20% soluble powder (Merck, Sharpe & Dohme) against coccidia were used. Frequent faecal examinations were made to check persistent infestation and treatment was repeated as necessary before the experiments commenced. Antibiotics were also used occasionally in non-specific conditions. Calves were identified by numbered ear tags.

2.2.2. Guinea pigs. Adult males and females weighing 500 to 900 grams were obtained from the Veterinary Research Laboratories, Kabete, Kenya. They were maintained on proprietary guinea pig pellets supplemented with cabbage or grass and were accommodated in

groups of four or five in wire cages. Losses associated with progressive wasting and inappetance and haemorrhagic enteritis occurred but were reduced by administration of neomycin-pectin suspension (Neopect-Assia, Tel Aviv). The guinea pigs were identified by ear-clips and raddle stick marks.

2.3. TICKS

The original stock of Rhipicephalus appendiculatus adults, (laboratory batch numbers 297/1838; 168, 195, 200, 243, 245, 246, 248, 250, 252/1853), was obtained from the tick colony maintained at the former East African Veterinary Research Organisation (EAVRO), Muguga, Kenya. This strain was obtained locally from cattle in 1952 and had been maintained ever since entirely on rabbits at EAVRO using the methods of Bailey (1960). Branagan (1974) confirmed that up to that date this strain showed identical feeding performance to those freshly obtained from the field.

In the present study the ticks were still maintained according to Bailey's methods but with some modifications. i) Calves were used exclusively as hosts and all engorged and moulted/hatched instars were maintained at room temperature (R.T.^o) (19^o to 23^oC) over moist sand in metal canisters. ('instar' refers to the individual developmental stages of larva, nymph and adult). ii) During the cold season, around 12^oC, an electric light bulb

(60 watt) was used to warm the cardboard hood containing the canisters up to 27°C as the development became unduly prolonged. This was discontinued, however, after a considerable loss of larvae - probably through desiccation, iii) Discarded plastic syringe packets holding fifteen to thirtyfive engorged females were used for oviposition and hatching. The bags (packets) were closed by stapling and ventilated by slits cut in one side. The packets were kept over moist sand until the larvae hatched. As soon as the larvae started crawling they were dispensed into 50x12.5mm tubes plugged with cotton wool. Better hatching rates were obtained than transferring the eggs into fresh tubes for hatching.

Batches of about 500 engorged larvae and 200 to 250 engorged nymphs were incubated and stored until required in 50x12.5mm and 75x25mm glass tubes respectively. After moulting and hatching all instars were allowed at least two weeks to harden before use. Failure to moult or death soon after moulting reduced the numbers of ticks, especially in batches collected from secondary infestations.

2.4. HOST ACCOMMODATION AND RESTRAINT

During the experiments calves were housed in 3.05m square stalls with cement faced walls and floor lit by large unglazed windows. The walls between stalls reached the roof. The stalls opened on to a cemented yard and the whole complex was protected by a water filled

moat on the outside. The stalls and the yard were cleaned out daily. The calves were kept separately during infestation to prevent mutual grooming and disturbance of the applied sleeves. However, the controls and those not being infested were kept in groups of three or five according to size. The infested calves were tethered by the head (or neck) and lightly hobbled to stop them grooming the infested ears. Alternatively they were held in wooden stanchions erected in some of the stalls. The intense irritation made some calves rub ears, even when lying down, on any raised object near the head. Thus shallow movable feed and water troughs were provided which made it difficult for the calves to rub against them.

2.4.1. Similarly the guinea pigs were caged individually during infestation and were prevented from gnawing at the body sleeve which contained the feeding ticks by means of a large cardboard ruff slipped over the neck and secured with zinc oxide plaster. They tended to react to the ruff during the first two days or so by pushing into the corners or between feed and water containers.

2.4.2. Maximum and minimum thermometers were installed in the stalls to record the ambient temperature (10° to 32°C). This was to monitor changes which

could influence the thermal regulation in young calves and obscure the direct tick effects. The low temperatures were recorded during the cold months of May/June to August.

2.5. TICK FEEDING APPLIANCE

Calico sleeves of two types were used for the application of ticks to the ears of calves and the trunk of guinea pigs. One type was plain with open ends and the other was a modification of the plain one. I modified the sleeve by fitting purse strings at either end and a zip along one side. The zipped sleeve facilitated inspection of the feeding site with minimal disturbance and made tick application to ^{the} trunk of guinea pigs and ears of calves much easier. Application of ticks through the zip window after sealing the fitted sleeve reduced losses during the process.

2.6. BLOOD SAMPLING

Blood samples, drawn by jugular venipuncture using 16 to 19G. hypodermic needles, were collected in ethylene diaminetetraacetate (EDTA) in 5ml bijou bottles and 20ml plain universal bottles as appropriate. The samples were processed on the same day except in few unavoidable circumstances. The following haematological and biochemical parameters were measured.

2.6.1. Haematology: packed cell volume (PCV), total red blood cell counts (RBC), haemoglobin concentration (Hb), total and differential white blood cell counts (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), plasma protein (TP), thrombocytes and reticulocytes.

Packed cell volumes were determined by heparinised microhaematocrit capillary tubes spun in an MSE haematocrit centrifuge and read against the Hawksley scale. Plasma protein levels were estimated in a refractometer¹ using the plasma in the haematocrit tubes. Red blood cell and WBC counts were made in a Coulter counter (model Z_B) set at amplification 0.1, aperture ¹/4, threshold 7-9, using "isoton" solution as diluent. Haemoglobin levels were measured in a Coulter haemoglobino-meter (model HGBR) using "zapo-globin" lyser. Mean corpuscular volumes were read in a Coulter MCV (model HCT) and the MCHC derived using the standard formula (Benjamin, 1969). The presence of reticulocytes was occasionally checked using supra-vital staining with new methylene blue. The check was done only whenever the total RBC counts dropped below $4 \times 10^{12}/l$ level. Moreover, reticulocytes in ruminants are normally associated with an anaemia crisis in contrast with guinea pigs where they are encountered fairly often (Schalm et al., 1975). Thrombocyte count estimations were done following standard haematological procedures using 1%

1 Cherwell Laboratories Limited, Bicester Oxon.
OX6 7XB, U.K.

ammonium oxalate solution as the diluent (Faulkner & King, 1970). For the differential count usually 100 white blood cells were counted.

2.6.2. Biochemistry: serum protein (S.P.) estimations and albumin determinations were made according to the methods of Reinhold (1953). The recording of the results for albumin estimations was incomplete and therefore omitted due to a mix up of figures and loss of samples. The globulins were separated from the albumin at 3000rpm in MSE centrifuge (type 4611 - 104) and the final reading taken in an Eppendorf photometer² with optical density (OD) measured at 546nm. The immunoglobulins (IG) were determined by the zinc sulphate test (Kunkel, 1947), OD readings were similarly made in the Eppendorf photometer and the results were recorded in turbidity units (T.U.):

$$\frac{\text{OD Test} - \text{OD control}}{\text{OD BaCl}_2 \text{ solution}} \times 20 = \propto \text{T.U.}$$

Serum glucose (S.G.) levels were determined by the Coulter Electronics glucose-kit³ using the hexokinase principle and read as before.

2.6.3. The haematological parameters recorded followed the routine procedure in our clinical laboratory and were facilitated by adequate equipment provided. The range of parameters offered a good basis for assessing the

2 Eppendorf Geratebau, West Germany.

3 Coulter Reagents Incorporated, New York.

alterations in the various indices. However, the biochemical estimations were restricted by availability and cost of reagents, time required to run certain tests, physical facilities in the laboratory catering for several services. Thus the compromise settled for SP., IG and SG estimations. The changes in total serum protein would represent direct feeding effects, and the IG should reflect the host response to the salivary secretion immunogen(s). However, albumin is considered a better parameter than S.P. because of the inverse response in globulins which masks the loss (O'Kelly et al., 1971). The serum glucose levels should indicate the metabolic changes which can be mediated through intoxication or reduced feed intake. A lot of work has been done on S.P. and IG. levels (Irwin, 1974; Penhale et al., 1970), however there is relatively little data on S.G. levels in calves. Thus the investigation of S.G. was speculative. Unfortunately samples could not be collected more frequently because of the limited facilities and the amount of work involved.

2.7. SPECIAL CYTOLOGY

While no special histology of the skin was planned cytology of the bleb (see C.8.1.7. C.10.4) reactions aimed at demonstrating the cellular elements recorded

by previous workers. It was therefore limited to simple microscopic preparations of fluid and scrapings collected.

2.8. URINALYSIS

Routine physical examination of the urinary system in calves involves visual assessment of the urine colour, volume, frequency and mode of urination. It is only when these characteristics are affected that more detailed analysis is indicated. Previous studies consulted do not record passage of abnormal urine in cattle infested with non-infected ticks. Thus the investigation was not considered beyond visual assessment in the design of the clinical examination. Nonetheless, by inference, the clinical pathological syndromes, such as haemorrhages, described by other workers led to anticipation of possible alteration in the urine picture. Consequently a close watch was kept and the frequency of urinalysis depended on the detection of changes. The infrequent urinalysis in the absence of gross changes was therefore speculative. The fresh samples collected were tested for changes in a) pH., b) specific gravity (sp.gr.), c) colour and clarity, d) presence of blood, e) ketones, f) protein, g) glucose and h) deposits. The reagents and equipment used were labstix⁴ (d, f, g), haemastix⁴ (d), acetest⁴ (e), refractometer (b) and light microscopy (h).

4 Ames Co. Stoke Poges Slough Bucks. England.

2.9. HISTOLOGY

Bouin's solution and 10% formol-saline were used for fixing and preserving tissues for future histological studies.

2.10. LYMPH NODE SIZES

Lymph node enlargement in cattle infected or uninfected with tick-borne diseases was reported but the magnitude of increase in size was not stated/ (Hewetson, 1971). Thus to obtain some idea as to what extent independent tick effects can provoke lymphadenopathy, measurement of sizes was undertaken. The measurements were predominantly made on the parotid nodes, being associated with drainage of the infested region and easily accessible. The anatomical position of the parotids could only permit measurement in two dimensions, length and width. However, other lymph nodes, such as the prescapular, situated in more loose tissues permitted a third dimension designated thickness. It should be stated that such measurements are not absolute as the skin and underlying connective tissue surrounding the nodes hamper accurate readings. The measurements were made by skin calipers of the type used in tuberculin testing.

2.11. HYPERSENSITIVITY RESPONSES

The rationale for investigating these responses is

stated under section 2.16. on secondary experiments. Materials used included female tick salivary gland extracts (SGE), immune sera obtained from calves and guinea pigs. It was not possible to collect pure saliva for technical reasons, time required for gathering sufficient volumes and limited adult tick supply. The salivary glands were obtained by dissecting three to four day fed female ticks and they were then homogenised in a tissue grinder. Feeding stimulates the salivary gland activity which enriches the required extract. The resultant homogenate was extracted with 2.5ml phosphate buffered saline (PBS) or physiological saline solution (PSS) per average 100 glands. The suspensions in tubes, occasionally agitated, stood in a domestic refrigerator for twentyfour to fortyeight hours to allow maximum extraction. The supernatant was dispensed in 0.5ml aliquots and stored at -10°C until required. Penicillin-streptomycin mixture at 10,000 I.U. and 13mg respectively was added to each batch of extract for control of possible contaminants.

2.11.1. Blood for serum was collected from animals which had been infested at least twice. The serum was separated on that day, dispensed in 5ml aliquots and stored in a similar manner. The control serum was obtained by bleeding non-infested animals. The samples were handled in sterile glassware. The donor guinea pigs were deeply narcotised with pentobarbitone sodium

6% solution before complete exsanguination. The aliquots were thawed in a water bath at 37°C before inoculation using 0.65x25mm 23 GI needles.

2.12. TRANQUILLIZERS

Thiazine hydrochloride 2% (w/v) (Bayer) at 5 to 30 mg/kg intramuscularly (i.m.) in calves and pentobarbitone sodium injection diluted 1:10 at 0.5 to 1.0ml intraperitoneally (i.p.) in guinea pigs, were administered to facilitate tick application and attachment when sleeves were not used in experiments V1 (C.9.), V11.1 and V11.4. (C.10.2 & C.10.5).

2.13. ANTIHISTAMINES

No special preference was put on the drugs used though it was desirable to have long action and high potency to minimise handling animals. The two features are described for the drugs chosen. Promethazine hydrochloride (Phenergan, May & Baker) has been used before and its use would give comparison with past results. Besides phenergan has a sedative effect as well. Desire to achieve high therapeutic levels and difficulty of handling animals at night led to more frequent injections during the day but appearance of toxicity signs forced a reversal in the dosage rate. Riek (1962) and Tatchell and Bennett (1969) demonstrated the significance of histamine in the mediation of clinical-pathological responses to Boophilus microplus infestation in cattle.

In order to corroborate their findings it was decided to investigate whether similar responses to Rhipicephalus appendiculatus could be demonstrated. Thus, promethazine hydrochloride 2.5% and 5% (Phenergan, M & B) and tripelethamine hydrochloride 2% (Vetibenzamine - Ciba-Geigy) solutions were administered i.m. in both the calves and guinea pigs during/secondary experiment VII.3. (C.10.4).

2.14. ANTIBIOTICS

Tick feeding causes cutaneous wounds and necrosis which predispose to secondary microbial infection. The host tissue responses are partly induced by these two factors. Administration of antibiotics eliminates the microbial component and therefore facilitates better appraisal of any intoxication effects. Thus oxytetracycline hydrochloride injection and soluble powder formulations (Pfizer) and penicillin-dihydrostreptomycin (Combiotic, Pfizer) were administered prophylactically in both hosts in the secondary experiments VII.I. and VII.4. to elucidate the tick effects.

The choice of drugs was based on their spectrum of sensitive organisms, cost and supply and therapeutic index. The synergistic action between penicillin and dihydrostreptomycin has great advantage in gram-negative bacterial infection normally sensitive to the latter. Development of drug resistance is reduced by administration of high dosage rates but this is hampered if drugs

with narrow therapeutic index are used over a period. The course of administration was based on the feeding period for the individual instar applied. Such treatment would be indicated in cases of suspected viral attack to ward off secondary complications. Moreover, debilitated animals are vulnerable to non-specific infections even in the absence of severe cutaneous lesions.

2.15. EXPERIMENTAL DESIGN AND PROCEDURES

2.15.1. PRIMARY EXPERIMENTS

The calves were the only host used and were divided into several groups as shown in the design. The choice to use calves, instead of old animals, was influenced by cost for animals and feeds, available finance, accommodation and other handling facilities. They are a lot easier to handle and examine. However, Yeoman (1966) asserted that young calves were unsuitable hosts as they carried very few ticks compared with the adults kept under the natural environment. The reason for this phenomenon is not clear. This would invalidate use of calves. Nevertheless, a compromise was that the calves used were at least two months old, thoroughly washed before infestation and artificially exposed to ticks. The size (n) of the experimental groups was similarly influenced and in addition the laboratory facilities and possible calf mortality before use were considered. Besides, when the idea of using all three instars was mooted then tick numbers and their breeding was also seriously considered. Thus animals, time and facilities required for breeding an adequate tick population for big experimental groups were major constraints. Nevertheless, the aim of this study was to investigate immediate and chronic effects caused by heavy infestations acknowledging that previous workers observed that such infestations were responsible for deleterious effects.

2.15.2. The choice of guinea pigs as the next host was again influenced by the same constraints. They were only used in the secondary experiments. Their utilisation reduced cost on animals and feeds and accommodation was a lot easier. Thus larger numbers for replicates could be obtained. The guinea pig was found highly susceptible to salivary secretion from Ornithodoros savignyi (Howell, 1966) and Dermacentor spp. (Allen & Wikel, 1978). Despite these advantages they presented problems through their compulsive reaction to tick feeding and the tendency to mutilate the infested sites even after all ticks had detached. Furthermore tick performance was worse on them than on calves. Riek (1959) found them unsatisfactory in his studies on tick resistance. Therefore they were not a very suitable substitute for calves, but where ticks had established a valid illustration of circulatory disturbance was presented. The rabbit is more suitable but was not used because of rapid development of tick rejection (Bailey, 1960; Branagan, 1974) which would demand larger numbers for the required tests. Such numbers were not readily available and small batches at a time would have unduly delayed completion of the study.

2.15.3. Studies on Rhipicephalus appendiculatus, a three-host tick, show that different hosts can be parasitized by the three instars individually or concurrently but the documented information on the clinical-

pathological responses to such infestations in cattle is not sufficient (Yeoman, 1966). It was therefore decided to examine the responses to individual or concurrent infestations in calves. A maximum of ten exposures was adopted to demonstrate the chronic effects guided by my observations on the influence of spraying on cattle response to heavy tick infestation. Two groups of calves, sprayed and unsprayed, kept in infested paddocks were observed for period of up to six months. The spraying was done once a week using toxaphene. The unsprayed group, over several weeks, developed chronic dermatitis, persistent pyrexia, lymphadenopathy, anaemia and severe loss of body condition which culminated in some deaths. The calves carried high counts of engorged female ticks, Boophilus decoloratus and Rhipicephalus appendiculatus. Conversely the sprayed group was only mildly affected and carried very few engorged ticks. These observations are unpublished.

The duplication of the adult infestation regime aimed at comparing the effects of low and high density. The situation can arise where the tick population fluctuates and/or cleansing is not efficient as illustrated in my observations. This expanded investigation was also encouraged by the knowledge that adults feed longer, are much bigger in size and are thereby capable of inflicting more serious injury by saliva injection and blood sucking than an identical density of an immature instar.

Thus a bias toward adults was more liable to induce readily detectable symptoms. The determination of the density for the different infestation regimes was based on the tick supply and experience gathered from previous studies. While the numbers of adults for individual infestations applied to one site may appear excessive it should be stated that they included both male and female. However, the male to female ratio was not standardised but a conservative proportion of 50:50 was assumed. Moreover, males have just a slight feeding impact on the host (C.5.1.8). Because of the fluctuation in supply the density for separate infestations are presented in ranges in the design but an overall average for individual animals was 800 and 1300 ticks for low and high regime respectively. In support of these high numbers applied to ears of calves, Dr. M.P. Cunningham (personal communication) recorded more than 5000 female Rhipicephalus appendiculatus ticks per ear on a number of steers which had died of tick toxicosis. The ticks were at different stages of engorgement. This unanticipated massive infestation occurred during East Coast fever transmission experiments when animals were exposed to infected ticks in the paddocks. Infestations of this magnitude can be expected during seasonal variations in the adult population in the field (Newson, 1977; Yeoman, 1966). Whilst studies by Branagan (1969) and Irvin et al., (1973) on the optimal numbers for experimental transmission of East Coast fever provided the background information, the density of the nymphal infestations in my study depended on the tick supply.

Similarly the density of larval infestations depended on the tick supply and was influenced by the work of Riek (1956) who applied up to 100 000 larvae on individual cattle. The mean numbers successively applied in the individual instar regimes are shown in Figures 3.2., 3.3., 3.4. The numbers used for concurrent three instar infestations were influenced by tick supply and the age of calves. It is acknowledged that massive infestation can quickly kill very young calves. Thus death from effects of a single primary infestation was not expected in this design. On the other hand the guinea pigs in the secondary experiments were infested with relatively massive numbers to demonstrate possible lethal effects in both susceptible and previously sensitized hosts.

Under field conditions animals can be continuously infested daily in the presence of a sustained tick population and this causes an overlap in the feeding of different life cycles. In order to assess the feeding effects of a single developmental stage in the life cycle on a susceptible animal a minimum interval of one week between applications was adopted. This would allow complete feeding of the immature stages and the majority of the adults. To facilitate interpretation, the infestation regimes were applied to different animals. Each

instar was allowed to feed to complete engorgement. The engorged ticks collected in the sleeves were counted and weighed, except for the concurrent three instars exposures. Reacting tissues at the feeding sites create an unfavourable environment for the ticks; thus sufficient time for healing is necessary to permit proper tick feeding in successive applications. This was also achieved through infestation of alternate sites, such as ears in calves. The number stated for individual instar infestations was applied to one site, the exception being the concurrent three instars R. appendiculatus infestations. All calves had not been previously exposed to ticks before the experiments.

2.15.4. The experimental regimes based on individual and concurrent three instars Rhipicephalus appendiculatus infestations were designed as follows.

Experiment (I) constituted the uninfested control group of five calves aged 2 to 6 months when used. The calves were not restrained but were separately accommodated from the infested groups.

Experiment (II) involved successive infestations with low density adult Rhipicephalus appendiculatus using five calves 3 to 8 months old. Two hundred to 2000 ticks per infestation were applied to ears of every calf for six to ten complete fortnightly exposures. All ticks (male and female) for each infestation were applied to one site. The higher numbers were accommodated and

during primary infestations 600 to 900 engorged females were recovered from individual animals. The calves were restrained during infestations to control grooming but were released during breaks.

Experiment (III) investigated high density adult ticks in five calves aged 2 to 4 months. Five hundred to 2 500 ticks (male and female) were applied to the ears of every calf per infestation for five to ten complete successive weekly exposures. Similarly one site was used for each infestation and ticks fed to complete engorgement. Restraint was the same but when calves became extremely weak during exposure they were released. Daily physical examination was done and blood samples for laboratory estimations were collected once in each exposure.

Experiment (IV): investigated nymphal R. appendiculatus exposures in five calves aged 5 to 8 months. One thousand to 3 500 nymphs were applied to the ears of every calf per infestation for ten successive weekly exposures. The nymphs were also allowed to feed to complete engorgement. Restraint during infestations was applied. Systematic physical examinations and collection of blood samples for laboratory estimations were similarly undertaken. Since no deaths occurred as predicted the autopsies were performed on slaughtered calves on completion of infestations.

Experiment (V) involved responses to larval R. appendiculatus infestations in five calves aged 3 to 6 months. An estimated 10 000 to 50 000 larvae were applied to ears of every calf per infestation for ten successive weekly exposures. The batch for each infestation was also applied to one site (ear) on each calf. Identical procedures were followed for physical examinations, collection of samples and handling engorged larvae.

Experiment (VI) investigated responses to joint feeding effects of the three instars R. appendiculatus when applied concurrently to three calves aged $1\frac{1}{2}$ to $2\frac{1}{2}$ months. Five hundred to 1 500 adults (male and female), approximately 2 000 nymphs and an estimated 20 000 larvae were applied to every calf per infestation for two to three complete successive weekly exposures. The ticks were applied to several sites including the ears, head, neck and trunk and fed to complete engorgement in all instars. The tick application was facilitated by narcosis using a tranquillizer (Rompun, Bayer). Restraint, physical examination and sampling were applied as before but the engorged ticks were not collected due to the unsuitable design of standings in the stalls. Autopsies were performed on one dead and one slaughtered calf.

2.15.5. The planned maximum ten exposures for every calf was not achieved in all cases due to early death or other complications, such as bacterial abscesses. The use of alternate sites (ears) during individual instar regimes allowed healing of inflamed tissues before next exposure of the same site. This permitted proper tick feeding in the successive infestations providing the more subtle host responses did not inhibit that feeding. However, dispersal of infested sites in experiment (VI) relieved some of the intense injury from clustering at one site. It is noteworthy that despite heavy clustering over the ears, accompanied by a serious reaction, healing of lesions was rapid in the absence of complications. Thus the allowed interval and use of alternate ears facilitated proper tick feeding in successive exposures. The intervals between successive exposures were occasionally increased due to a shortage of ticks or suitable hosts, or if there was a severe cutaneous reaction.

The ticks were applied according to Bailey's methods with modifications. The plain sleeves with open ends were fastened round the base of the ear with zinc oxide tape and the distal end was sealed with tape or staples. The zipped sleeves were similarly fastened after drawing the purse string, but the distal end was closed either by the purse string alone or with additional tape seal against escape of larvae. Another tape seal was applied to the fastened zip window for the same purpose. On

later infestations especially, the fastened sleeve was supported by extra strapping round the base of the horn(s), or the jaw and forehead in the absence of big horns. This reinforcement was warranted because of the vigorous shaking of the ears and/or head and occasional grooming associated with induced irritation which readily dislodged lightly fastened sleeves.

Before the first infestation all calves were thoroughly washed with soap and rinsed with plain water on three consecutive days to remove residual ixodicide capable of inhibiting tick survival. Superfluous hair was also clipped from the areas to be infested except in experiment (VI) (concurrent instars infestations). Clipping provided^a clear field for observing tissue reactions at the feeding site. Initially the ticks were inserted into the sleeve in the storage tubes but later, because of breakages, the ticks were emptied straight out of the tubes into the sleeve. Emptying the tubes through the zipped window after sealing the distal end minimised losses if the animal shook the ear/head in the process. Where sleeves were not used the animals were tranquillized to facilitate attachment. In experiment (VI) ticks were applied to different parts of the body by sprinkling from the tube or by allowing them to crawl out. This was especially necessary in guinea pigs which, through compulsive and vigorous head shaking, threw ticks off as soon as they landed on the ear.

Capsules were not used because of lack of proper adhesive material to anchor them. Restraint devices were constantly checked to reduce tick losses through crushing and detachment as grooming due to pruritis could not be completely prevented. In placid or effectively restrained calves and guinea pigs the sleeves were either completely removed or were rolled back to the base of the ear after the attachment was complete. Alternatively only the distal end and/or the zip window were opened. The sleeves were restored or closed when detachment of the engorged ticks was imminent. This variation provided more natural conditions and facilitated aeration and blood circulation. It also reduced accumulation of sweat and heat caused by complete sealing of the sleeve which would stress both the ticks and host.

The tick performance was monitored through the mode of attachment, feeding and detachment, engorged weights and numbers, viability during and after feeding, and reproductive capacity.

2.15.6. PHYSICAL (CLINICAL) EXAMINATION AND COLLECTION OF SAMPLES AND TICKS

The examination involved clinical signs elicited in different systems but the details and frequency of the systematic inspection varied. The outline of the examination was as follows.

(1) Body condition was assessed frequently by observing the state of muscle cover on forelimbs and hindquarters, prominence of ribs, spines of scapulæ and vertebrae, and hip points, coat appearance (grain, lustre, firmness), hollowness of paralumbar fossae and eyes. Demeanour and physique were observed daily for signs of depression or brightness and changes in musculo-skeletal activity such as change in gait, posture and ability to move.

(2) The cardiovascular system was considered sensitive and therefore was frequently examined. The rectal temperature was recorded twice daily and pulse quality checked at least two or three times a week during infestations. Heat in the extremities (vasomotor tone) assessed by palpation was checked whenever appropriate. Evidence of oedema and erythema and the appearance of visible mucous membranes were assessed daily. Oedema and erythema were checked in the skin, especially round attachment sites. The mucosae were inspected for colour, degree of moisture and lustre. The expected changes were pallor or congestion, excessive secretions which could alter in consistency to mucoid, purulent or frothy. The rationale for rectal temperature record is further explained in C.4.1.2. and evaluation of pyrexia levels in C.5.1.2.

(3) The lymphatics, as described in section 2.10, involved superficial lymph nodes which were frequently inspected for enlargement by palpation and measured whenever appropriate. Where abscessation was suspected needle

explorations were aseptically made and, on confirmation, material was aspirated in^a/sterile syringe for bacteriological investigation. Whenever abscessation was diagnosed the animal was withdrawn from the experiment on the grounds that suppurative reactions can cause serious systemic complications. Such reactions are difficult to control depending on the aetiology, nature of tissue reaction and accessibility of lesions to drugs. The respiratory system being less vulnerable was not examined in detail often but was checked daily for cough and discharges. Signs of dyspnoea were anticipated in case of anaemia crisis.

(4) The digestive system was examined daily for appetite, thirst, oral-gastrointestinal function and quality of faeces. Disturbance of the system reflects in impairment of other systems, e.g. body condition and physique. The anticipated disturbance would affect rumen and intestinal function. Muscle atony predisposes to rumenostasis, bloat and other digestive upsets. Diarrhoea or constipation can readily occur in weakened animals. Bloat can cause rapid death if not attended promptly; therefore frequent daily inspection was necessary for debilitated calves. The urinary system was inspected for urination and urine quality as indicated in section 2.8. Where appropriate calves were induced to urinate at inspection to assess the urine appearance. Daily observations were made on the nervous system for signs of pain, pruritis (irritability) and associated complications.

However, paralysis and other central nervous symptoms were unexpected. (Demeanour, strictly belongs to this system as it reflects mental alertness).

(5) The integumentary system is important since it is here that ticks first assault the host and gross reactions to light infestations are predominantly seen. Besides, considerable histological studies have been carried out in different hosts exposed to other ticks. The features constantly inspected included inflammatory changes (erythema, wounds, exudation), necrosis, nodules, hypersensitivity responses (bleb formation and urticarial eruptions) and associated traumatization, and transformations (keratosis, pityriasis, alopecia, scarring and distortion).

The recorded parameters were basically derived from the physical examination format used in our clinics. Nonetheless, the clinical data recorded by previous workers gave more prominence to some than other parameters and it was partly on this basis that certain systems were examined in greater detail. Confirmation of such responses was a major factor but, in addition, it was considered appropriate to demonstrate others which were less explored. Because of the variation in responses of different parameters some systems were examined more frequently than others as illustrated in this section. This variability can be physiological, as shown in the body temperature between the a.m. and p.m. levels. Thus the rectal temperature was recorded twice daily. The mode of examination was influenced by the signs sought and their

clinical manifestations. An example is the subjective assessment of body condition, demeanour, physique and nervous system responses which were derived by visual appraisal.

2.15.7. Blood samples for clinical-pathological estimations, as described in C.2.6., were collected before the first exposure and thereafter once during each infestation. Fluid and scrapings from blebs on the ears were occasionally collected and processed for simple microscopic study as indicated in C.2.7. Lymph node aspirates, aseptically collected whenever abscessation was suspected, were examined bacteriologically. From time to time lymph node puncture smears were prepared for screening tick-borne disease, e.g. Theileria parva schizonts (causative protozoan of East Coast fever). Uncatheterized urine samples were collected more regularly on/^{the} appearance of gross discoloration, otherwise infrequent samples were analysed as described in C.2.8.

Replete nymphs and females were collected once daily and the larvae twice daily. Heavy detachments collected in the sleeves adversely affected the fragile engorged larvae if the collection was done only once over twentyfour hours. The engorged ticks collected were counted and weighed in small samples as soon as possible after dropping and the mean weights calculated. However, the larvae were very active and, being fragile, crushed easily; therefore they were stored two to three days to

facilitate safe handling. The derived recoveries and weights for individual engorged instars are presented in Figs. 3.1., 3.2., 3.3., 3.4; and 3.6, 3.7, 3.8 respectively. The percentage recoveries for nymphs and larvae are presented in Fig. 3.5; however, these were not derived for adults because of unstandardised sex ratio. After weighing, each instar was handled as described in C.2.3. The ticks from the concurrent three instars R. appendiculatus regime were not weighed and the recovery rates were very low as explained in C.2.15.4.

2.16. SECONDARY EXPERIMENTS

These experiments were designed to test the findings obtained in the primary experiments and corroborate some of the responses reported by previous workers. They were based on the hypotheses stated below. The secondary experiments were intended to be as simple as possible. The conception of the hypotheses was associated with several phenomena of clinical and immunological nature. These phenomena are principally mediated by the salivary secretion, hereafter called agent(s), injected during tick feeding.

2.16.1. Hypothesis (I) stated that, 'The agent(s) cannot provoke a clinical-pathological effect on primary exposure'. This was conceived by the observation that

some ticks would inflict either severe or apparently insignificant clinical injury and the latter may pass unnoticed. Though the very slight injury may not be significant it can nonetheless induce a more subtle and important immunological response. This clinically elusive response can be revealed by immunological tests. Massive infestation is not necessarily a function of such a response but the agent is an essential immunogen. Thus the design for the investigation included high and low agent(s) challenge provided by feeding ticks and inoculation of salivary gland extract (SGE) in guinea pigs. The two levels of exposure, aimed at producing severe and slight clinical responses. An in vivo immunological test represented by a cutaneous hypersensitivity reaction, such as the (cutaneous) anaphylaxis or an Arthus-type reaction, was an integral part of the experimental design. The test would show whether an immunological response can be induced. The salivary gland extract (SGE), as the antigen, was the equivalent of the tick salivary secretion which could not be obtained. The hypersensitivity state was tested with the SGE inoculated intradermally and feeding ticks. The response was assessed by the skin reaction at the sites treated. The features assessed were erythema, oedema, thickness of the skin fold and other signs associated with local vascular changes. The procedures adopted are described later (C.2.16.5).

According to Roitt (1974), the cutaneous anaphylaxis-type I reaction is maximal within thirty minutes after the intradermal challenge with antigen and subsides thereafter. The constraints, imposed by the facilities available and the procedures followed in the testing of the hypersensitivity state, did not permit observation of the development of the cutaneous anaphylaxis in this study. During my study the readings of the intradermal challenge test were delayed and were done after 30 minutes for SGE antigen and after three hours for feeding ticks. Thus the occurrence of the cutaneous anaphylaxis cannot be ruled out because the standard procedure for the test (Roitt, 1974) was varied and the reaction was not checked within the recommended time. However, Roitt (1974) states that there is evidence that an immediate anaphylaxis-type I response is mandatory for initiating the first stages of the Arthus reaction. In view of this explanation the subsequent description of the test used for the hypersensitivity state will refer to a response of an Arthus-type reaction.

2.16.2. Hypothesis (II) stated that, 'The agent(s) cannot produce a clinical-pathological effect that is passively transmissible by immune serum'. Previous

workers tried to demonstrate the effect of passively transferrable humoral antibodies on tick feeding but the results were inconclusive. Various antibodies, such as complement fixing and precipitating, to tick secretions were demonstrated by in vitro tests. In preventive medicine such a procedure provides solid but temporary protection against corresponding immunogenic agents. Failure to produce significant inhibition to tick feeding did not eliminate sensitization of the skin. In corroboration it was designed to demonstrate whether such antibodies to Rhipicephalus appendiculatus could not be fixed in the skin. Thus detection of such immunogens in the skin was based on an in vivo passive cutaneous Arthus reaction test. Due to pressure of time and shortage of reagents, a quantal approach was followed using a higher dosage rate than in conventional methods. The procedures for the passive Arthus reaction test are described later (C.2.16.7).

2.16.3. Hypothesis (III) stated that, 'The agent(s) cannot produce a local reaction of a type that can be blocked by antihistamines'. Sensitization to foreign protein causes a hypersensitivity reaction when the subject is challenged after some time. This response is associated with high histamine levels in plasma and other tissues (Code, 1939). The hypersensitivity can appear as systemic shock or a cutaneous reaction. Essentially an antigen-antibody reaction occurs that leads to release of histamine responsible for mediating the clinical manifestations.

Riek (1962) demonstrated elevated histamine levels in plasma of previously sensitized animals challenged with further tick infestation. A severe exudative dermatitis was produced. Subsequently Tatchell & Bennett (1969) demonstrated the effects of antihistamines on the clinical-pathological responses to Boophilus microplus in cattle. The primary results in the present study have revealed symptoms in repeatedly infested calves which can be associated with histamine release. Though responses in cattle to different ticks can vary, it would not be unreasonable therefore to presume similarly mediated effects developed in calves exposed to Rhipicephalus appendiculatus. Thus it was justifiable to apply this hypothesis to the Rhipicephalus appendiculatus - cattle system to test whether similar responses could be demonstrated. Since the release of histamine is commonly associated with antigen-antibody complexes it was decided to include passive sensitization as described above (C.2.16.2.) in the investigation of the hypothesis. The choice of the antihistamines used is discussed in section 2.13. and regime of administration is described later (C.2.16.7.).

2.16.4. Hypothesis (IV) stated that, 'There is no difference between the effects induced by primary and repeated exposures to the agent(s)'. Previous studies revealed that repeated infestations caused more severe host

and tick responses than primary exposures. These findings are corroborated in the present study whereby additional heavy tick infestations provoked more serious reactions in the calves. The tick performance was similarly affected. Thus the hypothesis aimed at testing whether the responses to primary and repeated exposures were not distinctly different.

Procedures followed were the same as for primary experiments but with variation. Before the experiment a proportion of the animals were tested for non-specific cutaneous hypersensitivity reaction. The calves were 3 to 8 months old when used and were reared and fed as described before (C.2.2.1.). The main materials collected were only a few haematological samples and ticks. The ticks were handled similarly as in the primary experiments, but were not weighed. Thus the design and procedures for the secondary experiments were as follows.

2.16.5. Experiment (VII.1.) involved hypothesis (I) (C.2.16.1) and included four subgroups. i) Three hundred to 1000 and 60 to 800 adult ticks (male and female) were applied to each of six calves and ten guinea pigs respectively. ii) Four thousand and 5000; 700 and approximately 5000 nymphs were applied to two calves and two guinea pigs respectively. iii) An estimated 10 000 and 50 000; 2000 to 20 000 larvae were applied on two calves and each of three guinea pigs respectively.

iv) Three to 5 x 0.1ml intradermal/subcutaneous (i.d./s.c.) salivary gland extract (SGE) inoculations were administered daily to each of nine guinea pigs. The inoculations were made at one site in order to demonstrate the direct effect of the agent(s) in the SGE on local tissues. It simulated the feeding period of ticks. In addition it was intended to investigate the immunogenic potential of the agent(s) in the SGE. Similar control inoculations for local effects were done with phosphate buffered saline (PBS) or physiological saline solution (PSS) at separate sites on the belly.

Oxytetracycline hydrochloride was administered prophylactically to some of the hosts during the exposure as described under C.2.14. The guinea pigs received 4 to 8mg soluble powder twice daily (as a drench) and the calves were injected with 200 to 300mg once daily.

The ticks were applied as described before, except that in one calf larvae were applied concurrently to both ears. This was done purposely to boost the nymphal tick bank. Ticks fed to complete engorgement. The physiopathological and tick responses were observed as before and in addition the hosts were tested for active cutaneous Arthus reaction at ten to fourteen days of exposure. The timing of the test was based on the findings of Roberts (1968a).

2.16.6. Experiment (VII.2) was designed to investigate hypothesis (II) (C.2.16.2). Passively mediated responses were effected with materials described under C.2.11. The indicator for the passively transferrable effects was the passive cutaneous Arthus reaction test (PCA). Homologous immune serum at 0.3 to 1.0ml per site was inoculated i.d. at several sites in five calves and two guinea pigs. All hosts were unsensitized to tick antigens before the test with SGE. However, a third guinea pig received bovine immune serum which provoked adverse local reaction. An interval of twelve to forty eight hours was allowed for absorption before the intradermal test with 0.05 to 0.2 ml SGE. The control inoculations included, non-immune serum and PBS and PSS sites which were also tested with SGE, and SGE at non-sensitized sites. The tests were read at different intervals commencing twenty to thirty minutes of i.d. SGE and the final reading taken four to six hours later. Nonetheless, the sites were further inspected for a number of days. The parameters assessed included erythema-oedema zone (diameter), skinfold thickness and duration of reaction. The extended observation of the sites revealed other degenerative changes (induration, epidermal necrosis associated with inoculated tick antigen) subsequent to the test.

2.16.7. Experiment (V11.3.) tested hypothesis (III) (C.2.16.3.) and had two sub-sections. The antihistaminic effect was tested both on passively and actively sensitized hosts to the agent(s). One calf and one guinea pig were passively sensitized and four calves and two guinea pigs were actively sensitized to the tick effects. Tripelennamine hydrochloride and promethazine hydrochloride were used at dosages which varied according to size and species of the host and are described in C.2.13. The excitants used in the test were SGE, larvae and nymphs. The sub-sections were as follows.

a) Response in passively sensitized hosts.

The sensitization procedure was the same as for experiment (V11.2) (C.2.16.6.). A pre-sensitization cutaneous test carried out was negative. Single doses of tripelennamine hydrochloride at 100mg for the calf and 7mg for the guinea pig were injected half and one and quarter hours respectively before the intradermal SGE test. However, transient side effects appeared in the guinea pig (inco-ordination, fasculation and excitability). The experiment was repeated in the calf the next day without tripelennamine therapy for confirmation. Similar readings as before were taken at varied intervals (C.10.4.3.).

b) Response in actively sensitized hosts.

Four calves and two guinea pigs had two to four and one and two previous exposures to tick agent(s) respectively.

The excitants were, SGE at 0.1ml in both hosts; 1500 nymphs or an estimated 15 000 to 20 000 larvae for each calf; 2,000 nymphs for the guinea pig. Control inoculations were either PBS/PSS and SGE. The SGE was also given before the antihistamine to determine whether the drug effect caused genuine response. The saline inocula contrasted the nocuous effect of the SGE.

A single dose of 140mg tripelennamine hydrochloride was given to calf (A) tested with i.d. SGE one and four hours later. The experiment was repeated after twenty-four hours without the antihistamine as control confirmation. Readings were taken as before, commencing thirty minutes after i.d. SGE and the test sites inspected for several days after. The extended observation was meant to seek the progressive changes which accompany strong antigen-antibody reactions, e.g. Arthus reaction or necrosis mediated through circulatory disturbance. Another calf (B) exposed to larvae received multiple doses of tripelennamine hydrochloride commencing with tick application. The successive doses were given five hourly and overnight on the first day and thereafter twice daily until engorgement. The larvae settled quickly, except a small proportion that wandered for up to ten hours. Further identical exposure without the antihistamine was made on the 8th day of the last challenge for confirmation.

Similarly single 9.0ml and 0.5ml promethazine hydrochloride doses were given to the third calf (C) and one guinea pig (C) respectively one hour before challenge with

i.d. SGE inoculation. However, the guinea pig dose was split into two parts injected one hour apart as a precaution against toxicity. During a further test with nymphs, the promethazine hydrochloride was administered to the fourth calf (D) at 4 to 6ml twice on the first day and to one guinea pig (D) at 0.2ml once a day. The first injections were given at the tick application and the second in the calf eight hours later. However, the calf (D) was not treated on the second day as symptoms of toxicity appeared (excitability, cutaneous tremors, dry muzzle, frothy saliva; restlessness and recumbency; tachycardia, reduced vasomotor tone and subnormal rectal temperature; dilated pupils; anorexia, constipation and tenesmus). The ticks were settled by the first day, even in the calf with side effects. Then the antihistamine ^{the} was given only once on/third day when the side effects had practically disappeared. The treatment was not continued on the 4th day as the ticks were engorging. The calf was subsequently challenged with nymphs without antihistamine four days after all ticks from the last exposure had detached. All animals challenged were examined daily for development of physiopathological responses and for tick performance as indicators of the effects of the antihistamine therapy.

2.16.8. Experiment (V11.4.) tested hypothesis (IV) (C.2.16.4). Two broad groups were tested, the first comprised all hosts in experiment (V11.1.) (C.2.16.5.) (ten calves and

twenty four guinea pigs) and the second consisted of thirteen calves and nineteen guinea pigs. Some were drawn from the survivors in experiment(VII.1). The first group represented animals subjected only to the primary exposures and the second comprised hosts exposed more than once to the tick agent(s). The repeated exposures, predominantly to live ticks, varied in density, frequency and instar between individual hosts. The primary exposures are as described in C.2.16.5. The mode of tick application was the same as for primary experiments (C.2.15.5). A proportion of the hosts received prophylactic antibiotic treatment of oxytetracycline or penicillin-dihydrostreptomycin suspension (Combiotic, Pfizer) at the recommended dosage. Daily injections or drench (oxytetracycline for guinea pigs) were given one day before exposure until complete engorgement. The treatment was for control of non-specific infections during infestation as described in C.2.14.

The animals were physically examined every day, recording the same clinical-pathological responses as for primary experiments. Some animals were further tested for cutaneous hypersensitivity on a quantal basis. Ticks were handled as described before though not weighed. Occasionally the tick viability was tested in parallel using previously unsensitized guinea pigs in anticipation of possible rejection, especially in hosts exposed several times. Such rejection occurred without apparent gross skin reaction in one calf/twenty four hours of larval

application. Similar rejection was repeated during two further daily applications. However, the parallel exposures in the susceptible guinea pigs were successful. Besides the few haematological samples, occasional smears of blood from detachment wounds and fluid/scraping from blebs were prepared to assess the cellular changes induced. Autopsies were performed on dead animals and gross changes recorded. The induced clinical-pathological responses and autopsy changes in the two groups were compared.

2.17.

AUTOPSIES

All animals which died and the few that were slaughtered during primary and secondary experiments were autopsied immediately and the gross pathological changes recorded. The slaughtered animals consisted of withdrawn complicated cases and survivors of the ten exposures. The slaughter was undertaken to gain more information on the visceral injury attributable to specific and complication effects of tick feeding in live animals. Specimens of different tissues were collected and preserved in either Bouin's solution or 10% formol-saline for future histological study. Autopsy findings give corroborative information on the induced clinical-pathological symptoms.

2.18. ANALYSIS OF THE RESULTS

Tick recoveries were presented graphically as means (\bar{x}) and standard deviations (\pm SD) (Figures 3.1. to 3.4.). The percentage (%) recovery for larvae and nymphs were similarly presented (Figure 3.5.). The percentages of females recovered were not shown because the sex ratio was not standardised. The weights of engorged ticks were expressed as means and ranges (Figures 3.6 to 3.8.).

The haematological and biochemical data were expressed as means and standard deviations and presented graphically (Figures, 4.2 to 4.13; 5.5 to 5.16; 6.5 to 6.15; 7.3 to 7.14; 8.3 to 8.14, 9.4 to 9.12). Analysis of variance (Snedcor & Cochran, 1965) was initially applied to the data and whenever significant differences arose the Duncan's New Multiple Range test (DNMRT) (Bliss, 1970) was used to resolve significant subsets. The statistical results were given in tables (4.1 to 4.23). Where appropriate the data was further subjected to regression and correlation analyses (p. 117, 175, 194, 254, 264).

CHAPTER 3

RESPONSES OF THE THREE INSTARS OF RHIPICEPHALUS APPENDICULATUS DURING SUCCESSIVE INFESTATION REGIMES APPLIED TO CALVES AND GUINEA PIGS.

3.1. ATTACHMENT

The results presented are related to observations made on the primary (II) to (VI) and secondary (VII) experiments. Ticks attached rapidly, with few exceptions, during the primary (first) infestations (applications) in both categories of experiments. The majority of ticks settled within six hours of application. When a delay occurred the ticks wandered and/or clung to hair or the sleeve folds for up to two days before either finally attaching or falling off. This behaviour was most noticeable on the calves and guinea pigs during experiments (VI) (concurrent three instar infestation of R. appendiculatus - C.2.15.4) and (VII) (secondary, based on hypotheses - C.2.16.). In experiment (VII) it appeared in three calves and four guinea pigs; one calf; and one guinea pig, infested with adults, nymphs and larvae respectively. Besides the initial delay in attachment, some ticks detached after varying intervals before reaching full engorgement. Some were found reattached at new sites. This was observed on two calves and six guinea pigs infested with adult instar.

This phenomenon was commonly observed during successive repeated (secondary) infestations. The delay or failure to attach occurred irrespective of the reaction status on the skin. Ticks on subjects showing no apparent skin reaction detached some two to three days after settling and travelled to new sites. Under severe skin reactions the surviving ticks were found clinging to hair and sleeve folds not matted with thick exudate. Thus the proportion of unsuccessful attachments was increased by ticks engulfed and trapped in the flowing and coagulated exudate. The clinging ticks eventually became exhausted and fell off in a weakened state during the next twelve to fifteen hours. The incidence of these reactions during successive repeated infestations is summarised in Tables 3.1.i & ii.

*Two of the calves had two and the third had nine previous exposures. One of the two calves was being treated with antihistamine unrelated to the administration made in the secondary experiment (Vll.3). The guinea pig had no previous tick experience. The administration of antihistamines boosts the number of ticks which attach and feed successfully on immune hosts. This response was shown in the study on antihistaminic effects by Tatchell & Bennett (1969). An identical response was demonstrated in the present study as described in C.10.4.3. & C.10.4.5. However, Bagnall & Rothwell, (1974) failed to inhibit host immune responses in guinea pigs infested with Ixodes holocyclus. In order to test the viability

TABLE 3.1.i

The incidence of delay and/or failure to attach and premature detachment of R. appendiculatus during successive infestations on calves and guinea pigs.

INSTAR	DELAY/ FAILURE IN ATTACHMENT		
	INFESTATION(S) AFFECTED	HOSTS	
		CALVES	GUINEA PIGS
Adult	1	8 (17)	2 (10)
	2	1	.
Nymph	1	5 (7)	1 (9)
	2	1	.
Larva	1	3 (7)	2 (5)
	2	1	.
	3	2	.
DETACHMENT/MIGRATION AND FAILURE TO REATTACH			
Adult	1	8 (17)	2 (10)
	2	2	.
	3	2	.
	6	1	.
Nymph	1	3 (7)	1 (9)
	2	1	.
Larva	1	1 (7)	. (5)
	3	1	.
	7	1	.

(oc) = exposed animals observed during different experiments.

TABLE 3.1.ii

The incidence of delay and/or failure to attach and premature detachment of R. appendiculatus during successive infestations on calves & guinea pigs.

INSTAR	ENTRAPMENT 24 to 48 HOURS OF APPLICATION		
	INFESTATION(S) AFFECTED	HOSTS	
		CALVES	GUINEA PIGS
Adult	1	1 (17)	.
	2	1	.
Nymph	1	1 (7)	.
Larva	1	2 (7)	.
	2	1	.
	4	1	.
	9	1	.
*REJECTION >90% 24 HOURS OF APPLICATION WITHOUT FRANK SKIN REACTION			
Nymph	1	.	1 (9)
Larva	1	3 (7)	.

*one calf exposed twice previously was concurrently under the antihistaminic administration and guinea pig had no previous tick experience. Description of testing the viability of tick batches and confirmation of the response is given in the text (p.102).

(3c) = exposed animals observed during different experiments.

of the tick batches, therefore, two more daily tick applications were made on each calf during the same treatment, and batches of larvae were applied to two susceptible guinea pigs. The further applications on the calves produced the same results, whereas a very good take occurred in the susceptible guinea pigs. Nonetheless, still further infestation of R. appendiculatus nymphs on the calves (without antihistamine therapy) was successful but a big proportion died in situ within two to three days of application when partially engorged'.

Overall, the adult instar showed the most reluctance to attach and the greatest tendency to migrate to new sites. They also showed the best survival rate under severe clinical hypersensitivity reactions (bleb formation and exudation) though the engorged size and weights were much reduced. They avoided the bald areas produced by previous infestations and clustered along the edges and commissure of the pinna.

Attachment, especially round the head and ears, on the guinea pigs was markedly disrupted by impulsive irritability causing powerful shaking of the head and neck. Unattached and loosely anchored ticks were therefore easily dislodged. The reaction was evinced by both susceptible and sensitized guinea pigs. However, detachment through grooming was most evident in repeatedly infested calves which showed pruritis.

Partially fed adult ticks removed from guinea pigs in extremis were applied to susceptible hosts in experiment (VII) because they were still capable of further feeding.

The transferred ticks attached within an hour and fed avidly. The infested guinea pigs died three/four days later before the female ticks became fully engorged. Autopsy revealed severe haemorrhages on the skin pelts. The capacity to transfer to new hosts was also noted in partially fed nymphs which spontaneously detached from dead guinea pigs and readily attacked the nearest animals in the next cages. This rapid transfer confirms the observations made by Irvin et al., (1973) during studies on Theileria parva transmission in cattle. On several occasions I was bitten by such adult and nymphal ticks during this study. Repeated bites provoked an instant itchiness immediately ticks started feeding and this facilitated early deticking. It was interesting to note that at first the attached ticks over my legs or back of neck were not detected until they were markedly engorged.

3.2. FEEDING AND RECOVERY

Following established attachment the ticks fed well, as will be described later, in both first and subsequent infestations, although in the latter host responses caused frequent disruption. The physiopathological responses disturbed the engorgement of nymphs and adults. The affected ticks which were partially engorged stopped feeding and eventually withered and died in situ. However, in severe otitis externa rapid degenerative changes round attachment sites disrupted normal tick feeding in

calves. The otitis was mostly associated with heavy clustering of the adult ticks. On a few occasions, under the stress of host reaction, larvae and nymphs detached prematurely. They were partially engorged and not the usual blue-black colour. Nevertheless, discoloured specimens were also found among fully engorged tick batches, especially the larvae. The proportion of such specimens was greatest in the first detachment and markedly decreased towards the end of the feeding period. Discolouration may be caused by inaccessibility of capillary bed or partial dilatation of vessels in the epidermis/dermis which would only permit leakage of lymph alone without erythrocytes. Another effect observed was the slow engorgement of adults applied to calves. In most cases they were the size of three to four day fed ticks despite having fed for six to eight days. Apparently such ticks did not look sturdy and took longer to complete feeding and detaching. In successful feeding the period to complete repletion and detachment was fairly constant, varying only within narrow limits for each instar. Records from other studies show similar or markedly different feeding periods to engorgement for individual Rhipicephalus appendiculatus instars as illustrated in Table 3.2. The extended times to engorgement are not explained in all cases but some of the causes given for the anomaly include density of infestation (crowding), environmental temperature and development of resistance

TABLE 3.2.

Comparison of the average time to engorgement of R. ap-
pendiculatus ticks applied to cattle, rabbits and guinea
pigs.

Author(s)	Average time (days) to engorge on rabbits		
	Females	Nymphs	Larvae
Branagan (1974)	9.04	5.88	4.58
Average time to engorge on cattle and guinea pigs**			
Branagan (1974)	8.20	8.53	6.16
" *	9.30	8.12	7.51
" *	9.36	7.30	5.36
Irvin <u>et al.</u> (1973)	9.80	5.90	.
Theiler (1921)	7.0	3.0-5.0	3.0-5.0
Lutu (this study)	5.50	3.50	3.50
" " **	8.0	5.50	3.50

* derived from two steers infested during a three-way
comparison trial based on ticks fed on rabbits.

to ticks in the host. However, in the present study identical densities were applied to calves showing immune responses but the period of feeding to engorgement did not alter appreciably. Overall my figures are much lower but compare well with those given by Theiler (1921). Since different hosts can vary markedly in their responses to infestations, even of one type of tick, then the data from rabbits may not fully account for the revealed anomaly. Besides, times for adults and nymphs fed on guinea pigs demonstrated the difference attributable to host species. The shorter time obtained in this study cannot be attributed to environmental temperature as identical results obtained at both cold and hot seasons of the year when the ambient temperature ranged between 10° to 32°C.

Further to the comparisons on periods to engorgement (Table 3.2) the mode of feeding in the present study showed the following periods for completion of engorgement. The detachment of fully engorged ticks started on average five to six days post infestation (dpi) for adults and three to four dpi for larvae and nymphs. Highest detachment numbers occurred one to three days later in the different instars. The adults and nymphs applied to guinea pigs took longer to engorge with detachment starting seven to nine days post infestation and five to six dpi respectively. However, similar delays were sometimes encountered during secondary (repeated) infestations applied to calves.

The average period between the first and last detachment for fully engorged ticks was, adults: three to five days on calves and four to five days on guinea pigs; nymphs: three to five days on calves and three to six days on guinea pigs; larvae: two to three days on calves and three to five days on guinea pigs. These periods are summarised as follows.

AVERAGE PERIOD (DAYS) FOR COMPLETE DETACHMENT
OF R. APPENDICULATUS

HOST	INSTAR		
	ADULT	NYMPH	LARVA
Calves	3 to 5	3 to 5	2 to 3
Guinea Pigs	4 to 5	3 to 6	3 to 5

3.2.1. The proportion of engorged ticks recovered from the hosts varied and did not show consistent levels during successive infestations. The mean numbers of applied and the recovered engorged ticks and standard deviations (SD) of the recoveries are presented in Figures 3.1., 3.2., 3.3. & 3.4. Generally the first infestation recoveries were much higher than from the successive repeated exposures. The reduced recoveries were attributable to frank and inapparent physiopathological and behavioural host responses. The physiopathological responses included exudation, oedema and epidermal necrosis and rejection with no apparent clinical manifestation and the behavioural responses related to grooming. The exudation, oedema and necrosis physically restricted tick feeding and where the

FIG. 3.1: Experiment (II)

Mean numbers of males and females applied and engorged females recovered during successive low density infestations of Rhipicephalus appendiculatus in five calves exposed fortnightly. (Figures for ninth and tenth infestation missing for technical reasons).

FIG. 3.2: Experiment (III)

Mean numbers of males and females applied and engorged females recovered during successive high density infestations of Rhipicephalus appendiculatus in five calves exposed weekly. (Death reduced number of calves after seventh infestation leaving one finally - illustrated by broken line).

FIG. 3-1

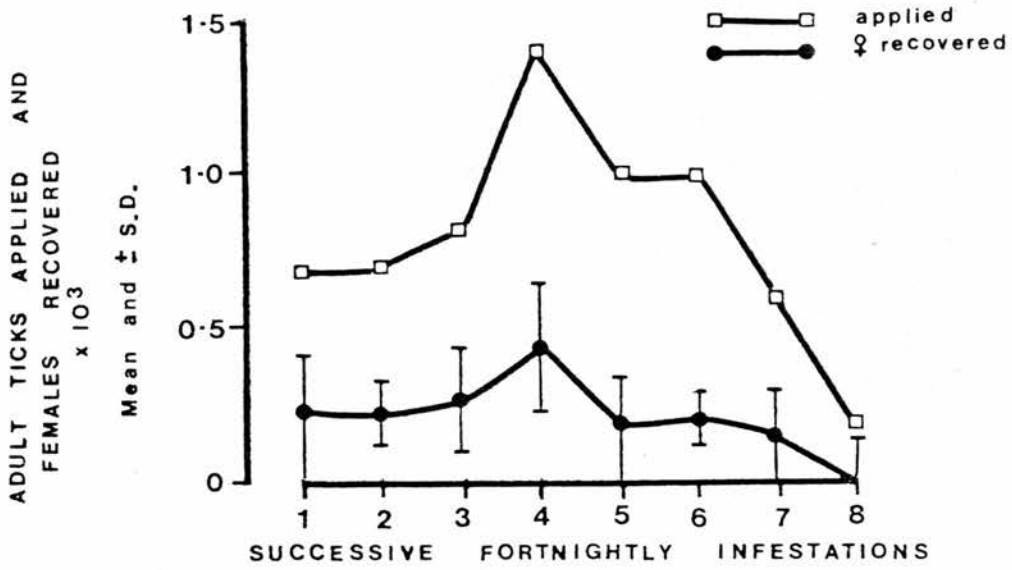


FIG. 3-2

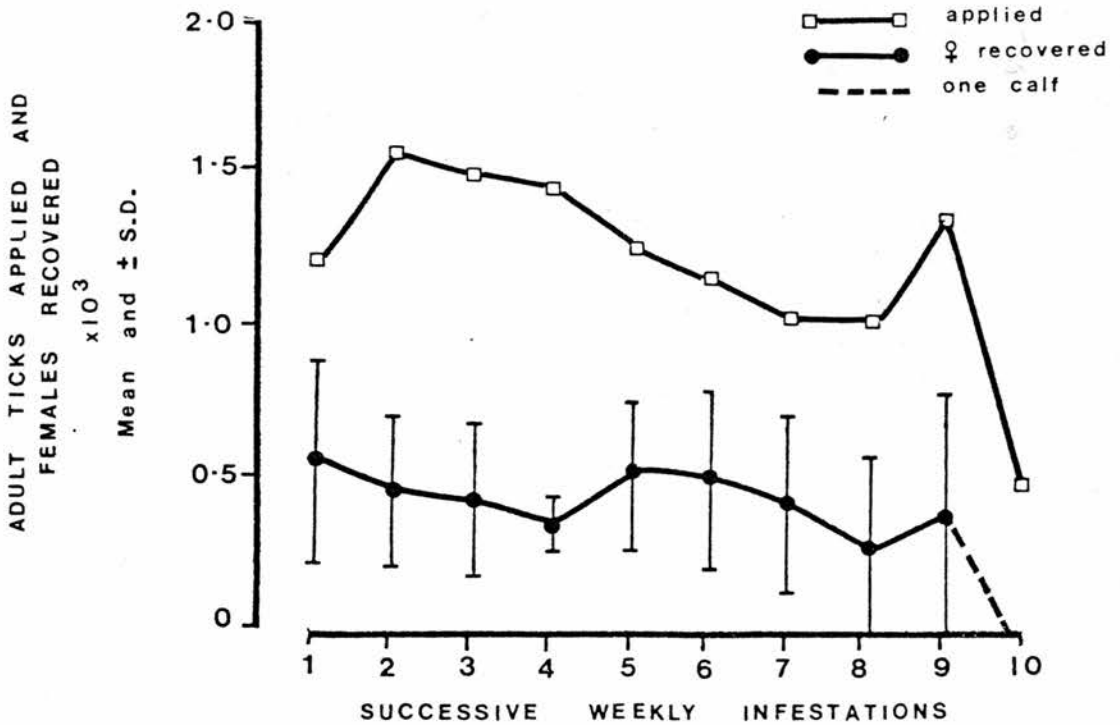
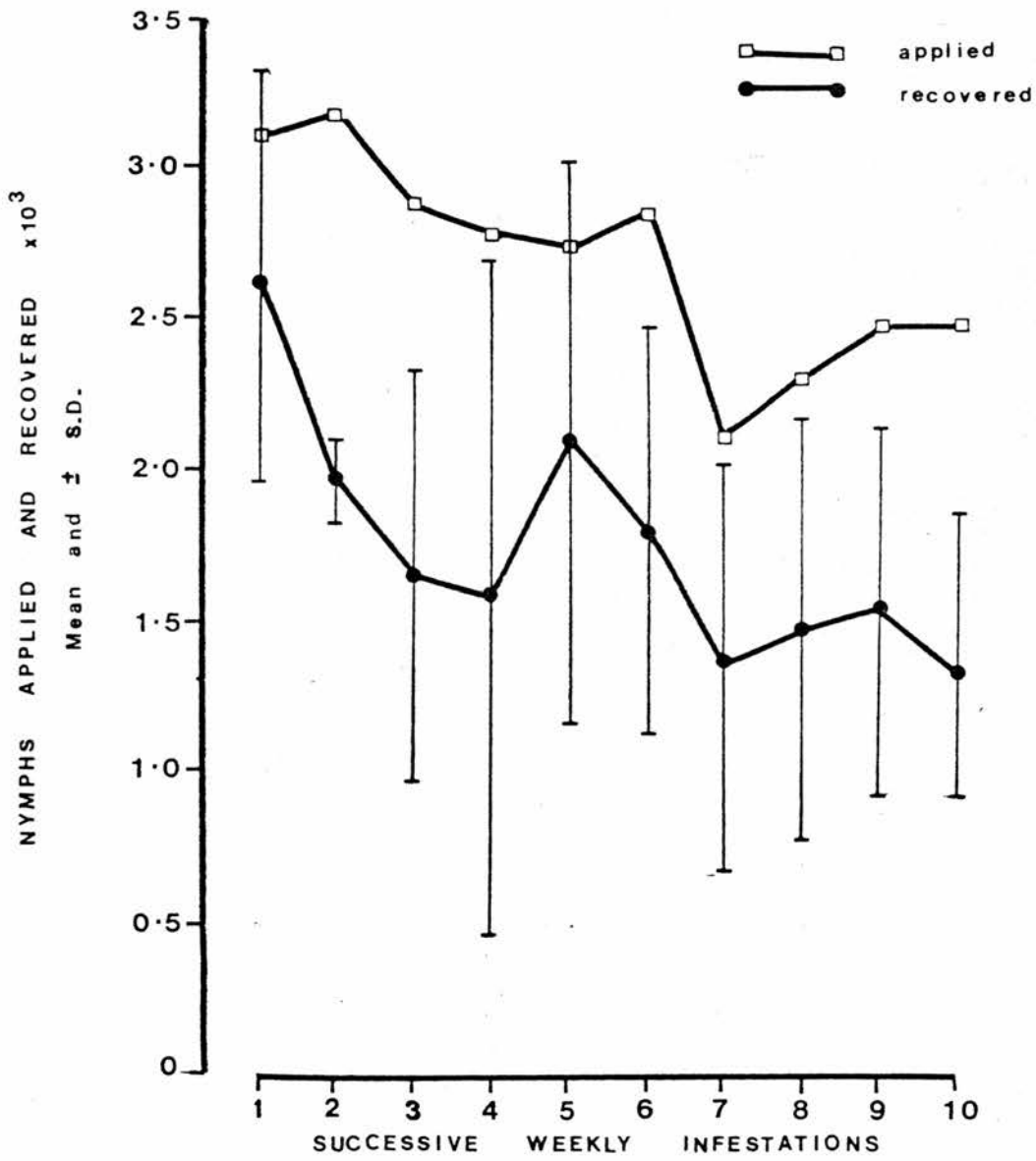


FIG. 3.3 : Experiment (IV)

Mean numbers of (unfed) applied and engorged nymphs recovered during successive infestations of Rhipicephalus appendiculatus in five calves exposed weekly.

FIG. 3-3



mouthparts were anchored in necrotic tissue no nutrients could be obtained and eventually led to death before complete repletion. Grooming, mediated through licking, rubbing and pawing (scratching) caused crushing and detachment. In the absence of the sleeve protection detached ticks were dispersed all over the stall. Vigorous shaking of head/ears, though strictly not described as grooming, caused appreciable losses during feeding and interfered with attachment when restraint was slack. In fact, this was the main reason for tranquillising animals in experiments (VI) and (VII) as ticks were being applied. In addition some adult ticks derived from nymphs fed on previously sensitized hosts failed to complete feeding because of reduced viability. The proportion of females recovered, shown in Figures 3.1. & 3.2. was also affected to some extent by the unstandardised sex ratio, thus the percentage (%) analysis was not applied to the instar. Besides, the males had very slight feeding impact on the host. This could be attributed to the interrupted mode of feeding while seeking to mate and the anatomy of the scutum which restricts the blood meal intake. In the absence of severe host responses the recovery rates of larvae (Fig. 3.4) and nymphs (Fig. 3.3) gave a standard with which to compare other situations. The percentage recovery rates of the immature ticks are presented in Figure 3.5. In certain individual calves infested with larvae there was almost complete failure of engorgement in the confines of the sleeve and the

FIG. 3.4: Experiment (V)

Mean numbers of (unfed) applied and engorged larvae recovered during successive infestations of Rhipicephalus appendiculatus in five calves exposed weekly.

FIG. 3.5: Experiments (IV) and (V)

Mean percentage recoveries of engorged larvae and nymphs from calves exposed weekly to successive infestations of Rhipicephalus appendiculatus. (Five calves per instar).

FIG. 3.4

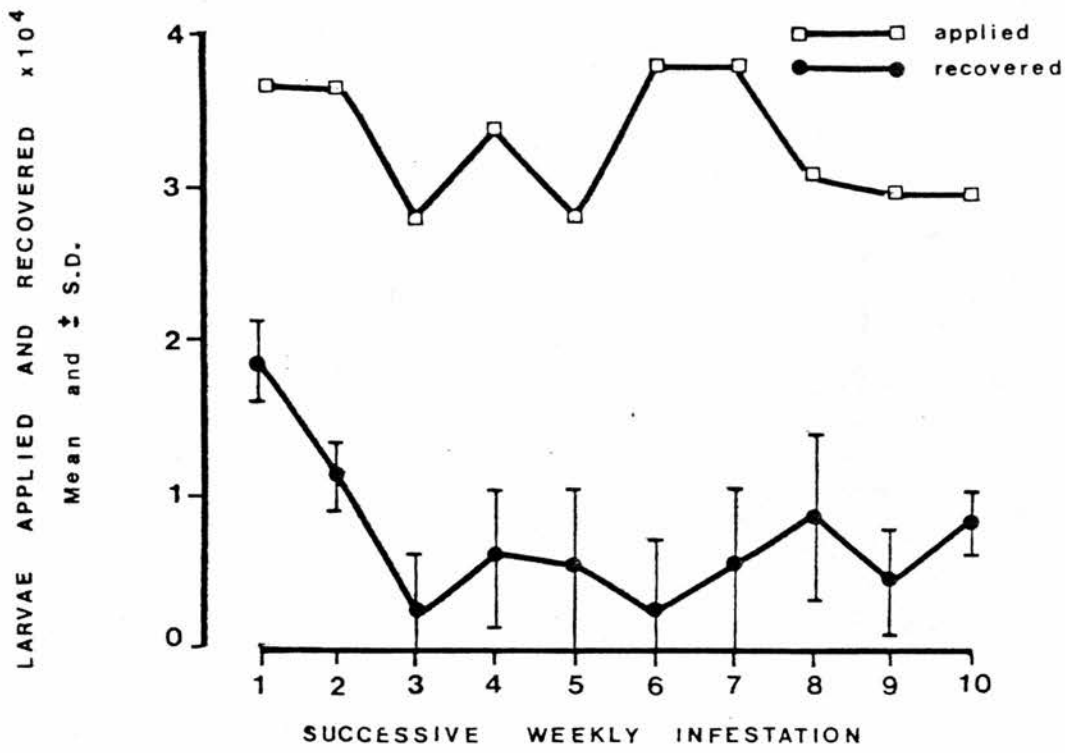
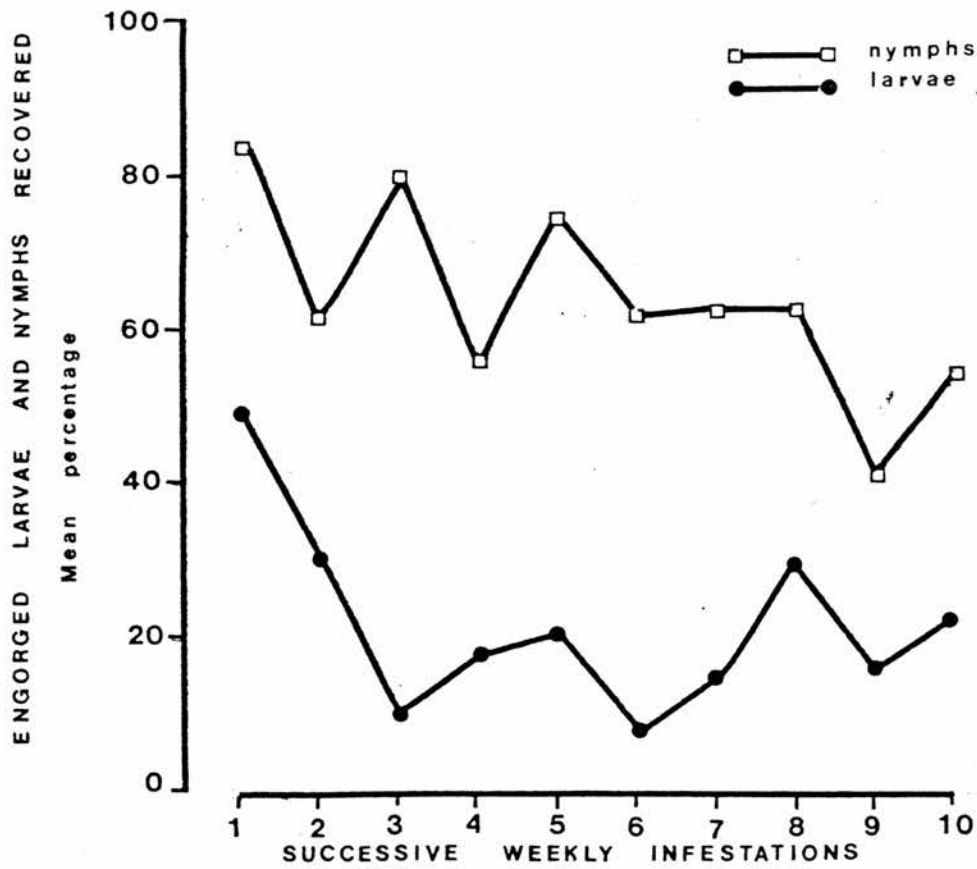


FIG. 3.5



recovery was rated 0.1%. However, administration of antihistamines reversed the results, as described in secondary experiments (C.10.4.3. & 10.4.5.). A proportion of these drug 'protected' larvae detached prematurely when partially engorged and were a light buff colour and wandered around. A sample of such ticks was collected and stored at room temperature for more than twelve days but did not moult and were still active. The partial meal, whilst inadequate for development, did sustain the energy needs of the larvae.

The mean numbers of engorged ticks recovered from successive infestations do not reveal a progressive decline in yield as would be associated with immune animals (Bailey, 1960). Establishment of a definite trend for Rhipicephalus appendiculatus fed on cattle would therefore require larger trials under standardised conditions.

3.3. ENGORGED SIZE AND WEIGHTS OF TICKS.

Generally the largest and heaviest ticks were recorded from first infestations as shown in Figs. 3.6, 3.7 & 3.8. However, this was not always true as revealed in the mean weights of adults and larvae presented in Figs. 3.6 and 3.8 respectively. Complementary to the increase in female engorged weights was the much lower proportion recovered (Fig. 3.2). The peak mean weights of the larvae (Fig. 3.8) apparently coincided with the lowest depression in the mean percentage recovery (Fig. 3.5). The variation in the mean weights

FIG. 3.6 : Experiment (III)

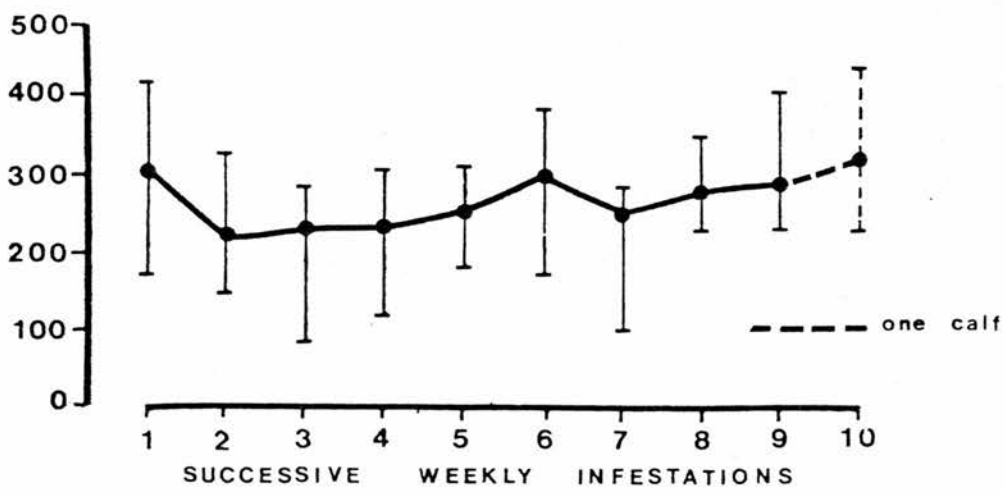
Mean and range weights of engorged females recovered from five calves exposed weekly to successive high density infestations of Rhipicephalus appendiculatus. (Death reduced number of calves after seventh infestation leaving one finally).

FIG. 3.7 : Experiment (IV)

Mean and range weights of engorged nymphs recovered from five calves exposed weekly to successive infestations of Rhipicephalus appendiculatus. (Single calf was sampled during latter infestations for technical reasons).

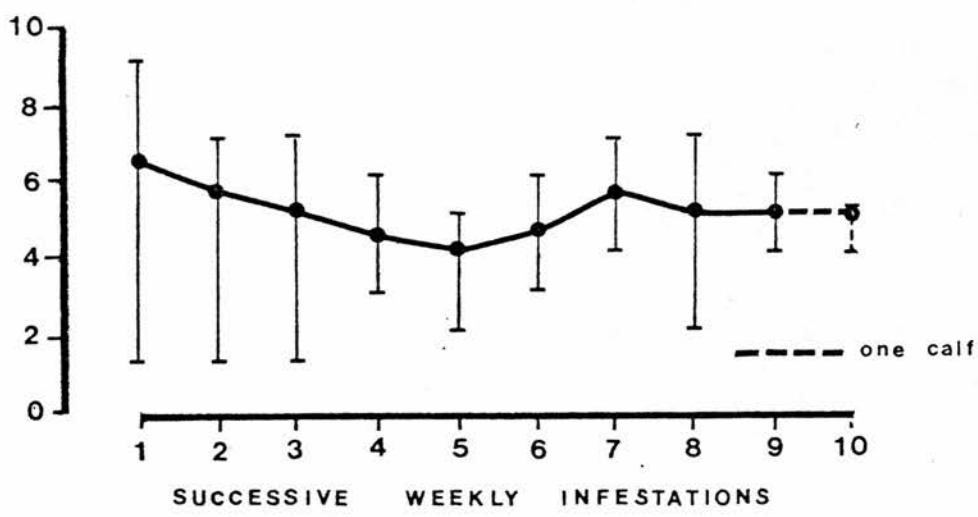
ENGORGED FEMALE WEIGHT mg

FIG. 3.6



ENGORGED NYMPHAL WEIGHT mg

FIG. 3.7



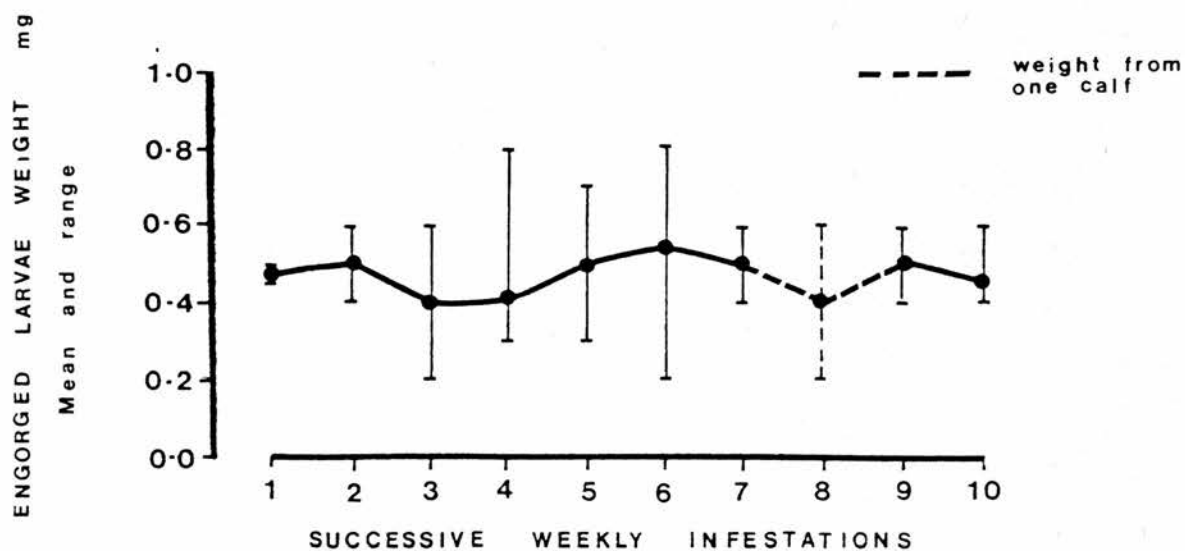
showed^a/definite trend through successive infestations and this was more consistent in the adults and nymphs. The trend showed a gentle decline which subsequently rose toward the original level. The larvae showed a different pattern and also presented a compact convergence of the mean and range weights during the first infestation (Fig. 3.8). The convergence demonstrated marked uniformity in unfed size and feeding capacity of the applied batches. It should be repeated that larvae were not weighed on the same day of detachment and this possibly influenced the figures obtained. Loss in weight in larvae occurs rapidly after detachment (Kemp et al., 1976). Thus the variation in the mean weights of adults and nymphs could be partly attributed to difference in sizes of unfed ticks. Existence of undersized ticks was noted in unfed adults and nymphs but the proportions were not established. Thus the mean weights of the engorged ticks did not show significant regression during the successive infestations ($r = -0.089$, $p > 0.05$). Further inspection of the results revealed that the mean weights and percentage recoveries of immature instars were not significantly correlated ($r = 0$, $p > 0.05$).

Similarly the mean weights and mean numbers of engorged females recovered did not show significant correlation ($r = 0$, $p > 0.05$). The phenotypic size of the engorged ticks fed on guinea pigs was comparable to

FIG. 3.8: Experiment (V)

Mean and range weights of engorged larvae recovered from five calves exposed weekly to successive infestations of Rhipicephalus appendiculatus. (Single calf was sampled during latter infestations for technical reasons).

FIG. 3-8



those fed on calves for the immature instars but differed for adult ticks which were generally much smaller. The mean weights from the primary (first) infestations though lower compare favourably with results obtained by Branagan (1974) during East Coast fever transmission studies. My figures are above the accepted minimum level of normality illustrated in data of ticks fed on rabbits (Branagan 1974). However, Irvin et al., (1973) recorded higher figures during the same studies. These results are compared in Table 3.3. Branagan (1974) observed appreciable progressive decline in mean weights of R. appendiculatus instars fed on rabbits during successive monthly infestations as shown in the table. Unfortunately ^{the} his figures for/corresponding regime of infestation in cattle are not available. The results thus compared were from single infestations on previously exposed steers and these infestations were not repeated in the same hosts. However, they serve to illustrate weight levels from primary (first) and repeat infestations. The mean engorged weights from successive infestations in my study do partly reflect the response demonstrated by Branagan in ticks fed on rabbits. Nevertheless, the trend was not stable in my results as during the second half of exposure the weights rose. It is unlikely ^{that} /repletion of established nymphs and adults was then significantly inhibited by host responses which were quite serious in several infestations. Whilst the

TABLE 3.3.
Comparison of mean engorged weights of R. appendiculatus fed on rabbits and cattle during primary (first) and secondary (repeated) exposures.

Exposure	MEAN ENGORGED WEIGHT (mg) ON RABBITS				Author(s)
	Females	Nymphs	Larvae		
First	325.00	8.60	0.50	Branagan (1974)	
Second	265.00	7.70	0.44		
Third	181.40	5.40	0.36		
(+)	260.00	7.00	0.40		
MEAN ENGORGED WEIGHT (mg) ON CATTLE					
First	362.90	8.70	0.56	Branagan (1974)	
"	445.90	9.40	.		Irvin et al., (1973)
"	314.20	7.50	0.49		
Secondary	367.60	9.00	0.53	Branagan	
	372.50	9.10	0.53	"	
*	246.83	5.90	0.47	Lutu	
**	284.53	6.10	0.46	"	

Mean weights on rabbits were randomly taken during routine infestations for maintenance of laboratory colony of R. appendiculatus. (+) reckoned the accepted minimum level of normality. Samples for first exposure on cattle were taken similarly (Branagan). Number of secondary exposures before the recorded weights not stated. Secondary exposures (Lutu) numbered up to nine at an average of week interval. * derived from first half and ** from second half of the experiments.

levels of repletion were not adversely affected a large proportion of ticks engorged on immune calves were unviable subsequently. This major variation between rabbits and cattle infested with R. appendiculatus could be attributed to species differences in response to ticks. It is possible that the rabbits produce marked and persistent immune responses to tick salivary secretions; and that the resultant resistance is more readily exhibited than would be the case with the bovine. The comparisons cannot be extended as Branagan himself regretted being unable to demonstrate this phenomenon in the bovine host due to lack of suitable hosts. However, workers in Australia have reported significant regression in the mean engorged weights of female Boophilus microplus fed on resistant cattle. In review the calves in my study did not produce persistent responses which had high suppressive effect on feeding ticks during repeated infestations of the three instars of R. appendiculatus. Moreover, Bennett (1975), Kemp et al., (1976) Kemp (1978) have demonstrated that once attachment is established the resistance factors do not influence the subsequent feeding. Furthermore the three-way comparative studies carried out by Branagan (1974) showed that cattle were satisfactory hosts, judged by engorged weights. In addition, Branagan/⁽¹⁹⁷⁴⁾reported that decline in yields and engorged weights were observed after repeated infestations during transmission experiments of Theileria parva, a protozoan causing East Coast Fever in cattle, but were

not of significant degree. Thus, in the absence of a more tangible explanation, it can be asserted that the pattern of engorged weights of R. appendiculatus fed on repeatedly exposed calves is attributable to the cattle being suitable hosts and lack of host immune influence on repletion once tick feeding is established.

3.4. VIABILITY DURING AND AFTER FEEDING.

In the primary (first) infestations the mortality in established feeding was generally very low. However, certain individual hosts yielded a relatively low percentage of engorged ticks and the cause was not apparent as in the initial phase of infestation the clusters were dense. The subsequent infestations were accompanied by much higher mortality in situ and after detachment. The toll varied between individuals during the different infestations. The proportion of death in situ was highest when there was entrapment in the exudate within twenty four hours of the infestation, especially in the larvae. In the absence of this phenomenon deaths were observed two days or more after attachment. Losses during the first exposures would conform to the observations of Bennett (1975) that the phenomenon is continuous throughout the life cycle of B. microplus. However, it is difficult to comprehend the loss of individual instars of R. appendiculatus applied for the first time on susceptible cattle. The ticks which were active,

light coloured, partially fed and stunted, withered and died. When dropped they were often completely dried or flaked off with the peeling epidermis. On the other hand ticks which had fed normally and attained good size sometimes turned darker and became inactive. On detachment they became black and died. This was more noticeable in the adults and nymphs. Similarly some ticks, normally developed, lost their gloss and died three to four days after detachment. Sometimes such ticks became mottled or brown, shrivelled and died before oviposition. The nymphs affected similarly failed to moult.

3.4.1. On the whole female ticks engorged on susceptible guinea pigs died in proportionately greater numbers than those fed on previously exposed calves. The majority were mottled and shrivelled and laid very small egg batches or none. It was estimated that over 50% of the engorged females recovered were not viable. The immature instars were less affected though occasionally between 25 to 47% of well fed nymphs were found unviable on detachment.

Severe otitis externa under heavy clustering in some calves arrested growth and caused death of ticks at different stages of repletion. This was aggravated by rapid decomposition of lymph and blood from detachment wounds. The otitis occurred mostly during infestations / ^{with} adult R. appendiculatus.

Engorged immature instars from first infestations had very good moulting rates. Under suitable conditions, ^{the} temperature and humidity (though/latter was not measured), over 50% of the viable ticks moulted in two weeks after detachment. However, subsequent infestations showed lower and more variable rates of moulting. Nonetheless, larvae which fed successfully produced consistently high moulting rates. Conversely, 5 to 30% of the viable nymphs from a number of secondary exposures failed to moult or the adults emerged from them were of impaired vitality and died after a short period. The phenomenon was recorded in five calves in experiment (IV) and can be summarised as follows.

Table 3:4 **

	SUCCESSIVE INFESTATION							
	1st	2	3	4	7	8	9	10th
Calves* affected	3	3	3	2	1	1	1	1

* 3 calves yielded such ticks on four occasions each.

3.5. FECUNDITY AND FERTILITY.

Female ticks engorged on calves during first infestations showed high reproductive capacity. This was reduced by varying degrees during subsequent exposures although the engorged female ticks appeared apparently normal. The abnormal characteristics were reduced egg batches including total failure to oviposit and poor or

** N.B. Incidence of unviable engorged nymphs recovered from five calves during successive infestations.

non hatchability. In some cases 30% of female ticks from individual recoveries failed to lay eggs. The ticks fed on guinea pigs produced very poor eggs or none at all. Most of these egg batches hatched poorly and some of the non viable ones changed colour and lost their gloss. The failures were predominantly associated with ticks fed on calves during secondary infestations. However, the ticks fed on guinea pigs were affected even in the primary infestations and their egg production was only quarter of that laid by ticks fed on calves. Thus the guinea pig was unsuitable for feeding ticks required for breeding.

3.6. EFFECTS OF HOST RESPONSES UPON TICK PERFORMANCE

The effects were most marked during secondary exposures in calves though ticks fed on guinea pigs were equally affected in the primary (first) infestations. The guinea pigs exerted their effects through behavioural and simple skin reactions and to greater extent through less explored responses. In calves the clinical hypersensitivity response in the form of bleb formation, manifested as pseudovesicles and pustules, had a drastic effect especially upon larvae. The attachment, feeding and viability of applied ticks were disturbed. Besides entrapment, the ticks which detached prematurely migrated to other sites but eventually became exhausted and died. Rejection within twenty four hours of application in the

absence of a gross skin reaction was observed. These inapparent physiopathological responses also inhibited proper feeding and viability which in turn disturbed the reproductive potential. The encrustations from excessive exudation and the under-run necrotic epidermis deprived ticks of nourishment and this eventually led to their death.

The calves groomed as a result of pruritis. In order to obtain relief from the intense irritation calves licked, pawed (scratched) and rubbed the infested areas and also vigorously shook the ears/head. The grooming crushed and led to premature detachment of ticks. The traumatization, where successful, created ^{an} unfavourable environment for proper feeding and survival of ticks. The guinea pigs showed impulsive irritability which provoked vigorous and frantic shaking of ears and head as soon as the ticks were applied. The shaking scattered any ticks which were not firmly attached. Besides they savagely scratched or bit at the infested areas. The attacked sites were severely denuded of skin and bled profusely and thus created unfavourable conditions for proper tick feeding.

3.7. SUMMARY OF SIGNIFICANT FINDINGS

Attachment was rapid, with few exceptional delays, during the first applications. Delays or failure to attach encountered in secondary exposures were commonly

associated with frank host tissue responses, e.g. hypersensitivity reactions, or inapparent responses and behavioural reactions e.g. rubbing. Detachment and migration to new sites some days after settling in both primary (first) and secondary exposures were observed and this mostly involved the adult R. appendiculatus. Antihistamines administered during applications of larvae and nymphs improved the attachment rates.

Established attachment was followed by good feeding which was often interrupted in secondary exposures. The interrupted feeding was accompanied by partial repletion and such ticks died in situ. Successful feeding was accompanied by fairly constant periods of engorgement and detachment in the three instars of R. appendiculatus. High recoveries were obtained in primary infestations but were varied and generally much lower during subsequent infestations. Total annihilation of applied larvae through clinical hypersensitivity reaction was common. Antihistamines boosted feeding and recovery rates. Primary infestations, with exception, produced ticks with greater engorged size and weights but the frequency of these features declined in the secondary exposures. The decline in the mean weights of engorged ticks did not show a significant regression during successive infestations. There was a recovery in mean weight levels during the latter exposures. R. appendiculatus females fed on guinea pigs were much smaller than ones fed on calves but the

immature instars were comparable.

Mortality was generally very low in established primary infestations, but was considerable in the secondary exposures through physiopathological and behavioural responses, e.g. entrapment in exudate and rubbing. Ticks died in situ or on detachment either partially or completely engorged. Viability was most adversely affected in ticks fed on guinea pigs and this was specially evident in female ticks. Moulting rates were higher in immature instars fed during primary than secondary infestations. Tick rejection through frank or inapparent physiopathological host responses, especially in secondary exposures, significantly depressed tick performance.

CHAPTER 4

EXPERIMENT (I): RESPONSES OF UNINFESTED CONTROL GROUP OF FIVE CALVES.

4.1. The calves, two to six months old, were kept in different stalls apart from the infested groups and were not restrained as grooming was of no consequence in their responses. They were sprayed at least once a week against possible attack from stray ticks and they were not previously exposed to ticks. Regular physical (clinical) examinations and collection of blood samples were undertaken. The blood samples for haematological and biochemical estimations were collected weekly. The physical examinations covered almost all accessible body systems but the details of the signs registered differed, e.g. inspection of cardiovascular function concentrated on daily (a.m. & p.m.) rectal temperature whereas the lymph nodes were inspected only twice a week. Thus the responses manifested were recorded under two main headings, viz: physical (clinical) signs and clinical-pathological findings. A similar pattern of description will be adopted for responses of other experimental regimes but will also include autopsies.

4.2. PHYSICAL (CLINICAL) SIGNS

The calves kept free of ticks remained healthy throughout the experiment in comparison with other groups

which evinced varied degrees of ill-health as a result of Rhipicephalus appendiculatus infestations. However, non-specific infections and other factors caused some disturbance in the normal health of the calves. The disturbance occurred at different times in individual calves, but the affected calves remained bright. The situation in the group could be summed up as follows.

The average responses, especially in pyrexia and gastrointestinal function, for the uninfested group were markedly influenced by two of the five calves. The two calves frequently exhibited elevated rectal temperature and digestive upset which were not associated with specific causes. Otherwise all the calves appeared bright and active. This adverse reaction occurred predominantly during the initial period of the experiment. The parameters investigated showed these findings.

4.2.1. The status of the body condition was quite good throughout despite digestive and pyrexia disturbances. The status was graded by visual assessment of the coat appearance (grain, lustre, firmness), prominence of bones (scapular and vertebral spines, ribs, hip points), muscle cover over hindquarters and upper forelimbs, and hollowness of paralumbar fossae and eyes. Weekly weights, as another means of assessment, were briefly taken but had to be abandoned because the

weighbridge was installed in an unsuitable place. Similarly the demeanour and physique of the calves remained bright and active. However, handling greatly excited the two calves, (singled out before) and invariably disturbed their cardiovascular and respiratory function which became accelerated whenever physical examination was undertaken.

4.2.2. Examination of the cardiovascular system entailed the assessment of rectal temperature and heat in the body extremities (hereafter termed vasomotor tone), heart and pulse qualities and appearance of the visible mucous membranes. The analysis of the rectal temperature taken twice daily (a.m. & p.m.) was based on a seven-day unit period. The unit period was the average time the female ticks took to become completely engorged. Adoption of the a.m. and p.m. measurements depended on the physiological diurnal variation in the normal rectal temperature at these times. The recording aimed at detecting marked changes in the range of normal temperatures, especially elevation above critical levels (hereafter termed pyrexia). Thus the means of weekly rectal temperatures were unsettled during the first four weeks (Fig. 4.1.). The elevated mean temperatures indicated a slight pyrexia (evaluation of pyrexia levels is described under C.5.1.3.). The critical level of thermal reaction was fixed at 39.3°C based on data

accumulated over three years in the indoor environment at the different seasons of the year on the same premises used for this study. The ambient temperature in the stalls was recorded and varied between 10°C and 32°C. The record was to monitor extremes which could possibly influence the heat regulation mechanism in calves, thus confounding the effects caused by the feeding of Rhipicephalus appendiculatus ticks. The means of daily rectal temperatures agreed with the means of the weekly rectal temperatures. The means of the daily rectal temperatures for some weeks are presented in Figures: 5.2 to 5.4., 6.2 to 6.4., 9.2 & 9.3, 7.2, 8.2, where comparisons are made with the response of the groups infested with instars of R. appendiculatus during corresponding periods. Heart and pulse activities assessed by auscultation with stethoscope and palpation respectively were generally found stable and undisturbed. Nonetheless, transitory elevation in the rates was constantly detected in the two excitable calves. The heart beats intensified and were heard standing beside the calves without auscultation. A similar reaction was obtained in one calf which developed a marked clinical hypersensitivity reaction to the larval infestations. The visible mucous membranes, viz: ocular, nasal, buccal (oral), vulva, were normal (pink, moist, glistening and intact) with just occasional slight ocular (lachrymation) and nasal serous discharges (secretions altered in quantity and consistency).

FIG. 4.1.

Mean weekly Rectal Temperatures of five uninfested control calves during experimental periods. (Arrow marks normal critical temperature).

FIG. 4.2.

Means and standard deviations of Packed Cell Volumes of five uninfested calves during experimental periods.

FIG. 4.1

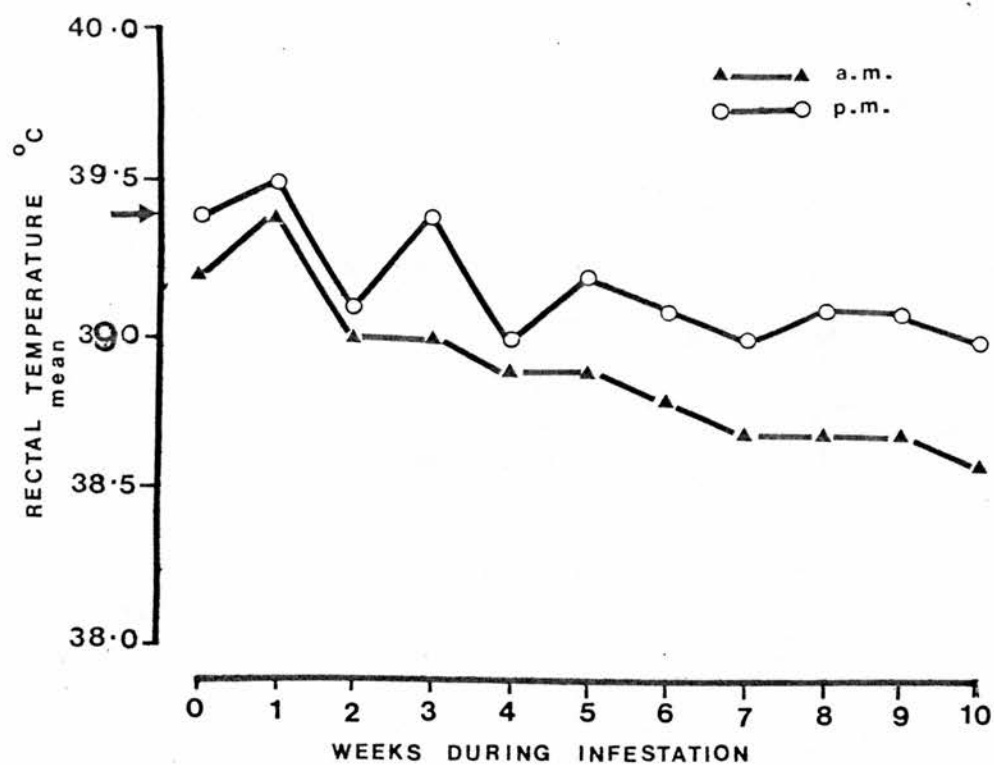
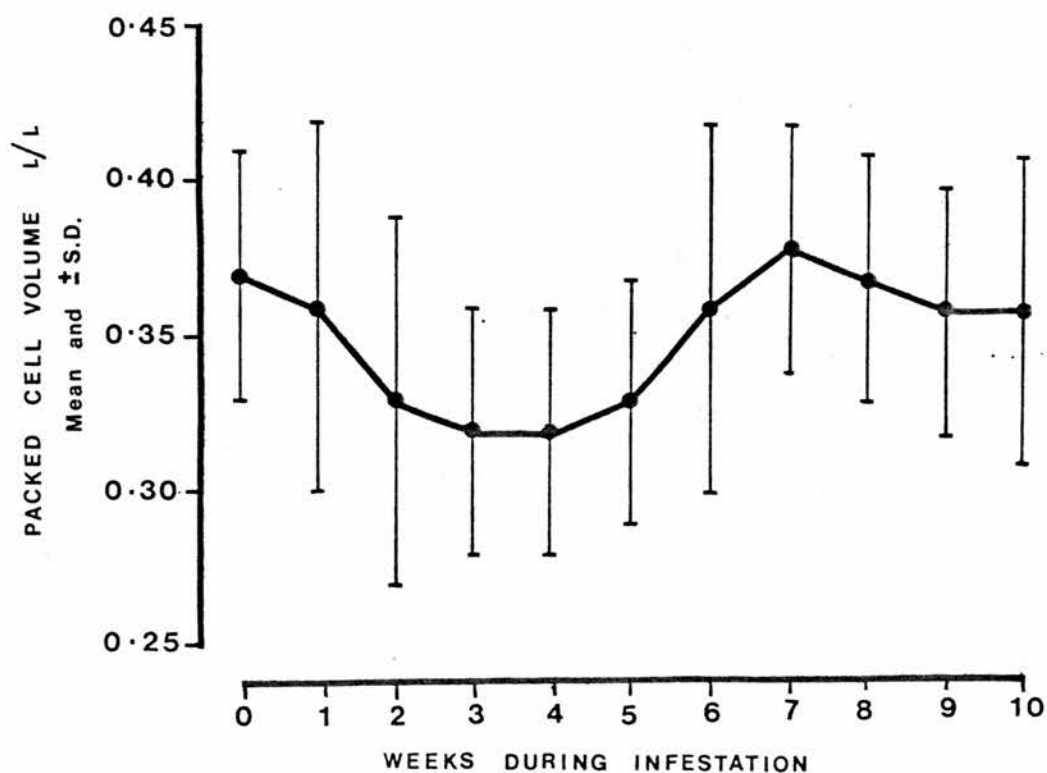


FIG. 4.2



4.2.3. The lymphatic system was unaffected and the superficial lymph nodes were just palpable. This status was distinctly at variance with the responses induced by R. appendiculatus infestations, especially of the adult instar, which provoked serious lymphadenopathy. Because of the dormant state the nodes were inspected infrequently.

4.2.4. The function of the respiratory system was stable but the excitable calves exhibited transitory accelerated respiratory rates and one calf developed a dry cough for some weeks. Occasional nasal discharge of serous nature and low quantity was observed. The gastrointestinal activity showed some upset but all calves maintained keen appetite throughout the experiment. Mild and brief attacks of bloat and diarrhoea occurred from time to time in individual calves and subsided with or without symptomatic treatment. In contrast with the infested experimental groups the uninfested calves exhibited no disturbance in other body systems which remained intact and functioned normally.

4.3. CLINICAL-PATHOLOGICAL FINDINGS

4.3.1. Haematological estimations.

The absolute values for the means of weekly samples and their standard deviations are presented in Figures 4.2 to 4.10. The variation in the mean values for erythrocytic indices (Figs. 4.2 to 4.6) were all within the normal ranges (Coles, 1974; Schalm et al., 1975). The mean corpuscular haemoglobin concentrations (MCHC) (Fig. 4.6) were the most stable but other indices showed appreciable fluctuation. Despite the variations in mid-experiment the final levels returned almost to original pre-infestation values. The ranges of the standard deviations were generally small, except for the packed cell volumes (PCV) (Fig. 4.2), the latter estimations of red blood cell counts (RBC) (Fig. 4.3) and mean corpuscular volumes (MCV) (Fig. 4.5). The mean levels at that stage showed an inverse relationship between RBC and MCV. But the marked fluctuation in the MCV levels cannot be explained as there was no critical decline in the red blood cell counts or packed cell volumes to provoke a response of such magnitude. Whilst the mean corpuscular volumes are associated with the response of packed cell volumes, the patterns for the two indices were not consistent. However, there was close similarity between estimations made at weeks 0 and 10, indicating stabilisation in the system. The thrombocyte estimations obtained were

FIG. 4.3

Means and standard deviations of Red Blood Cell counts of five uninfested control calves during experimental periods.

FIG. 4.4

Means and standard deviations of Haemoglobin Concentration of five uninfested control calves during experimental periods.

FIG. 4.3

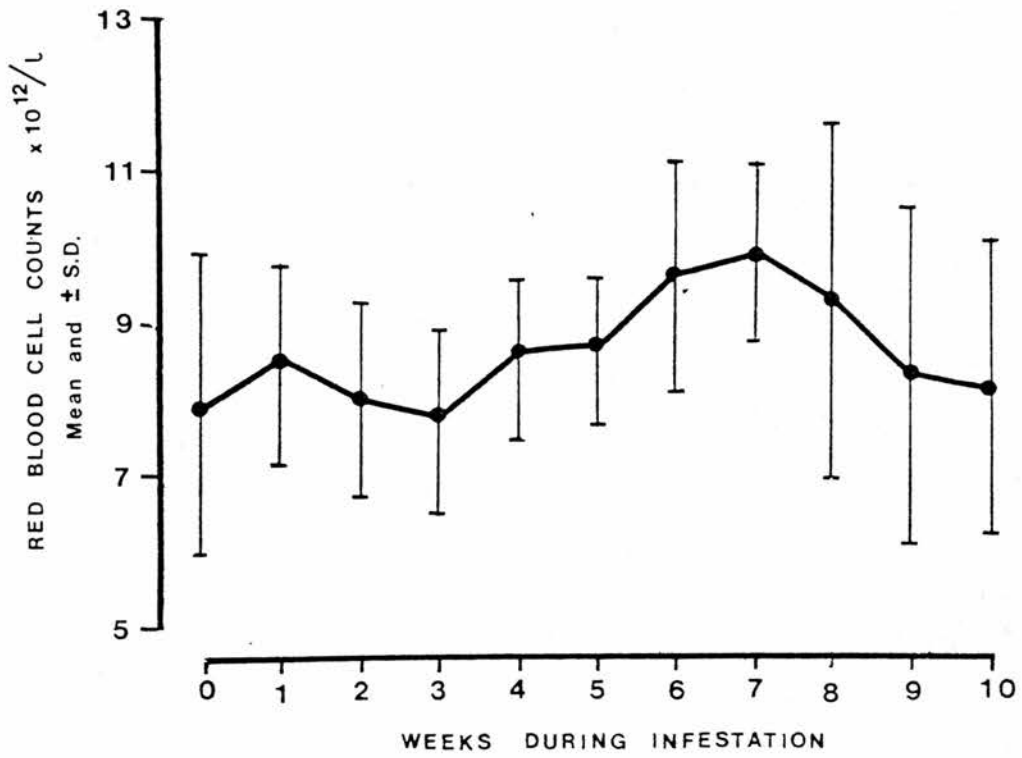


FIG. 4.4

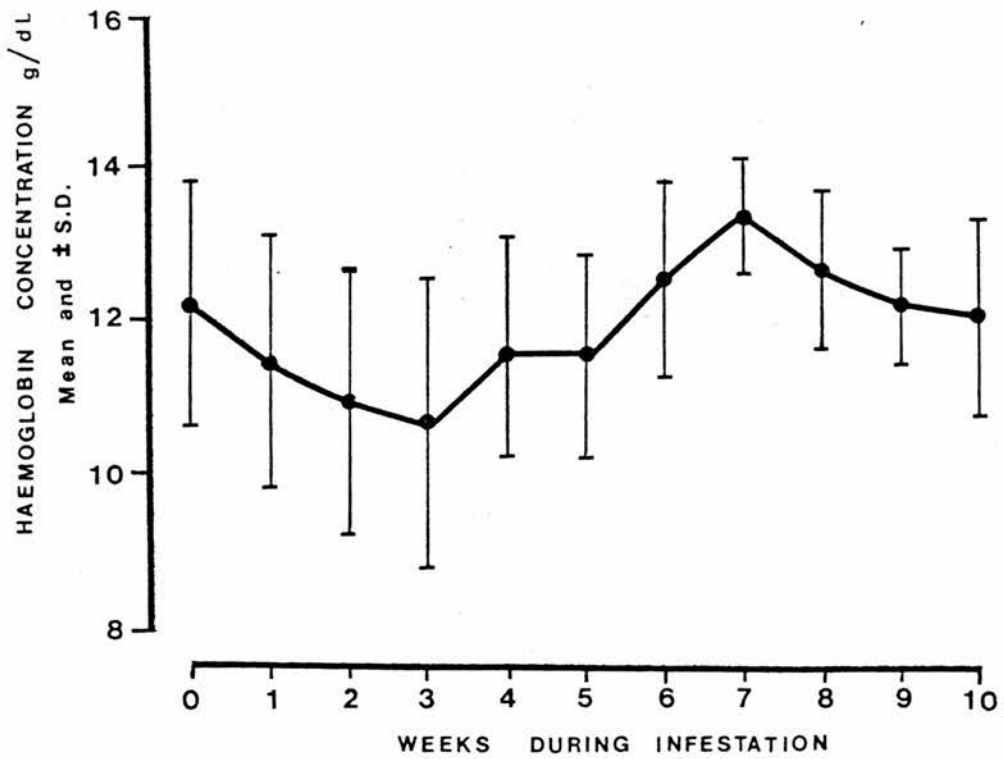


FIG. 4.5

Means and standard deviations of Mean Corpuscular Volumes of five uninfested control calves during experimental periods.

FIG. 4.6

Means and standard deviations of Mean Corpuscular Haemoglobin Concentrations of five uninfested control calves during experimental periods.

FIG. 4.5

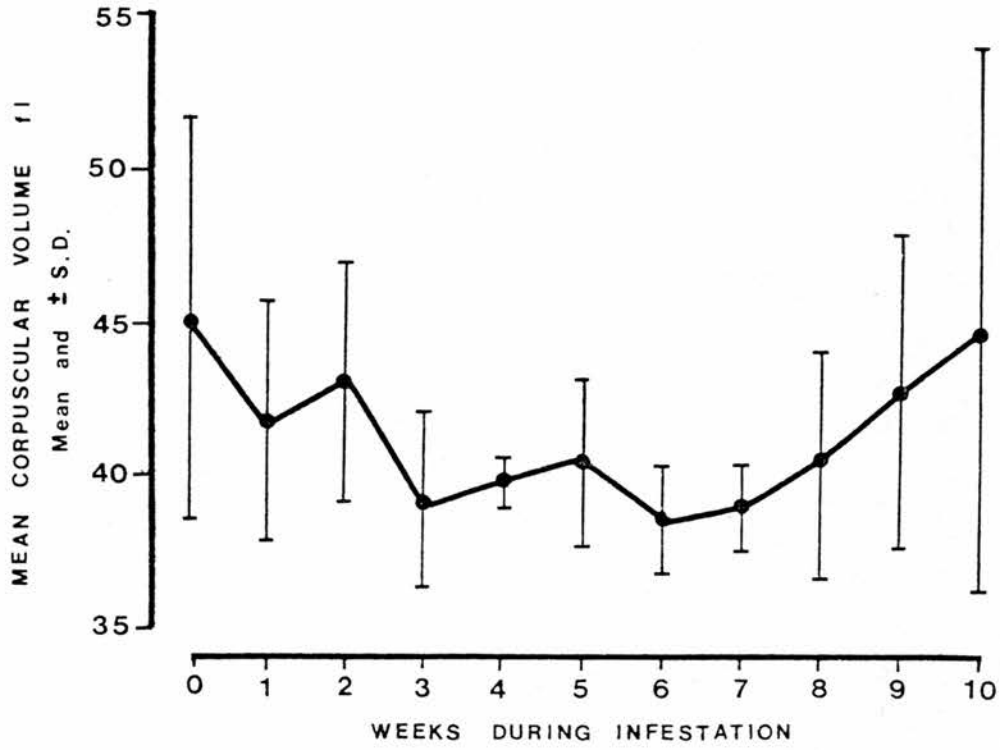
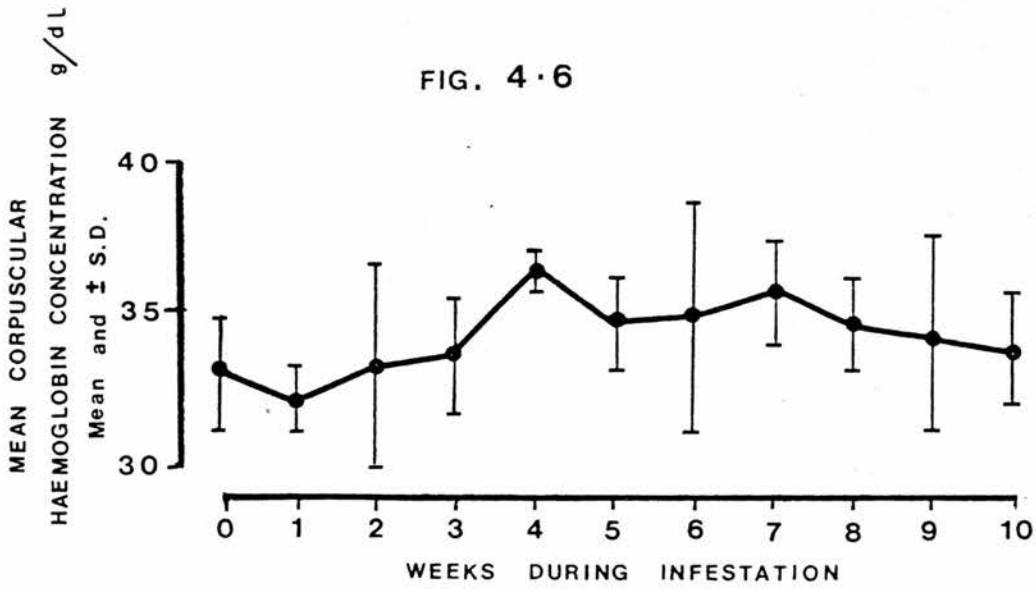


FIG. 4.6



unreliable due to difficulties associated with manual techniques and therefore were omitted from the results of all experimental groups investigated. Hence thrombocytes will not be mentioned again in subsequent chapters.

The upper mean values for all leucocytic series, except the eosinophils, slightly exceeded the normal levels in several weekly estimations (Figs. 4.7 to 4.10). The elevation in the means was mainly caused again by the two calves which showed leucocytosis for three and six weeks. The leucocytosis coincided with clinical manifestations (pyrexia, coughing, diarrhoea) indicative of microbial infection which was not established. Eosinophils were found in only 25% of all counts compared with 24 to 47% in the infested groups (C.5, 6, 7, 8 & 9). However, the absolute counts were low in all experimental groups which is not representative of responses to parasitism mediated through the skin. Nonetheless, Schalm et al., (1975) state that comparatively few eosinophils appear in circulation in health, yet the numbers are sufficient to reflect significant changes in the pathophysiology under certain conditions. They record that histamine is chemotactic for eosinophils, such that an elevated histamine level would attract eosinophils from bone marrow into the circulation. It is further argued that eosinophilia is not an expression of a single disease entity such as parasitism, but rather is an occurrence to be anticipated in a wide variety of

FIG. 4.7

Means and standard deviations of White Blood Cell counts of five uninfested control calves during experimental periods.

FIG. 4.8

Means and standard deviations of absolute Neutrophil Cell counts of five uninfested control calves during experimental periods.

FIG. 4·7

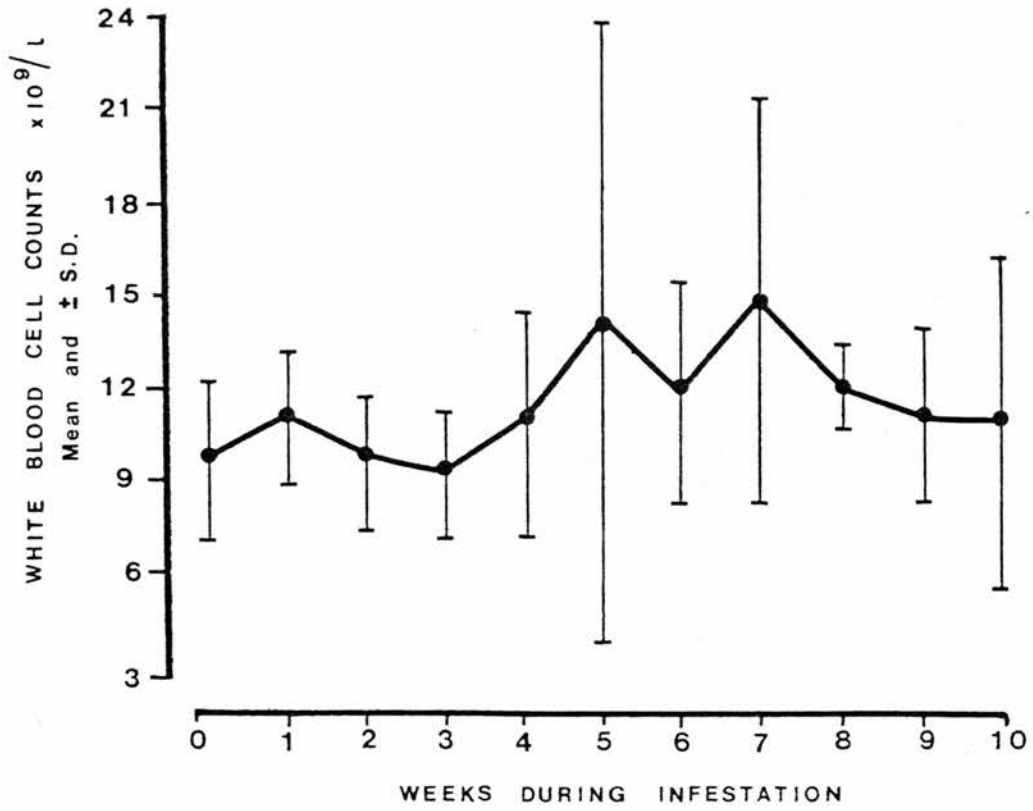


FIG. 4·8

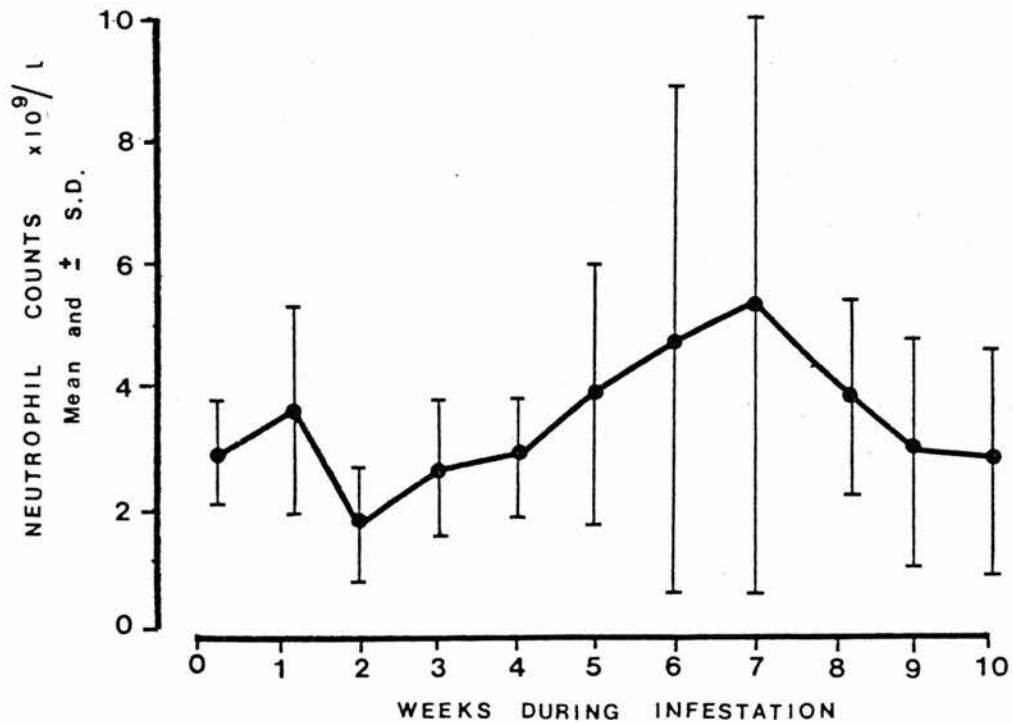


FIG. 4.9

Means and standard deviations of absolute Lymphocyte Cell counts of five uninfested control calves during experimental periods.

FIG. 4.10

Means and standard deviations of absolute Eosinophil Cell counts of five uninfested control calves during experimental periods.

FIG. 4·9

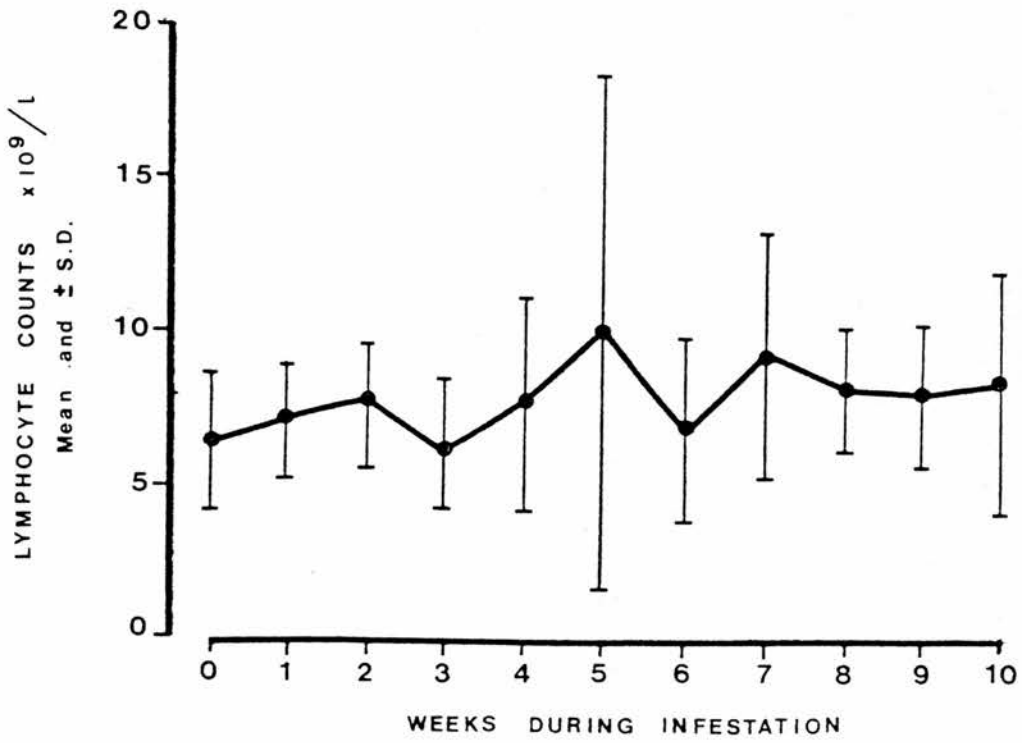
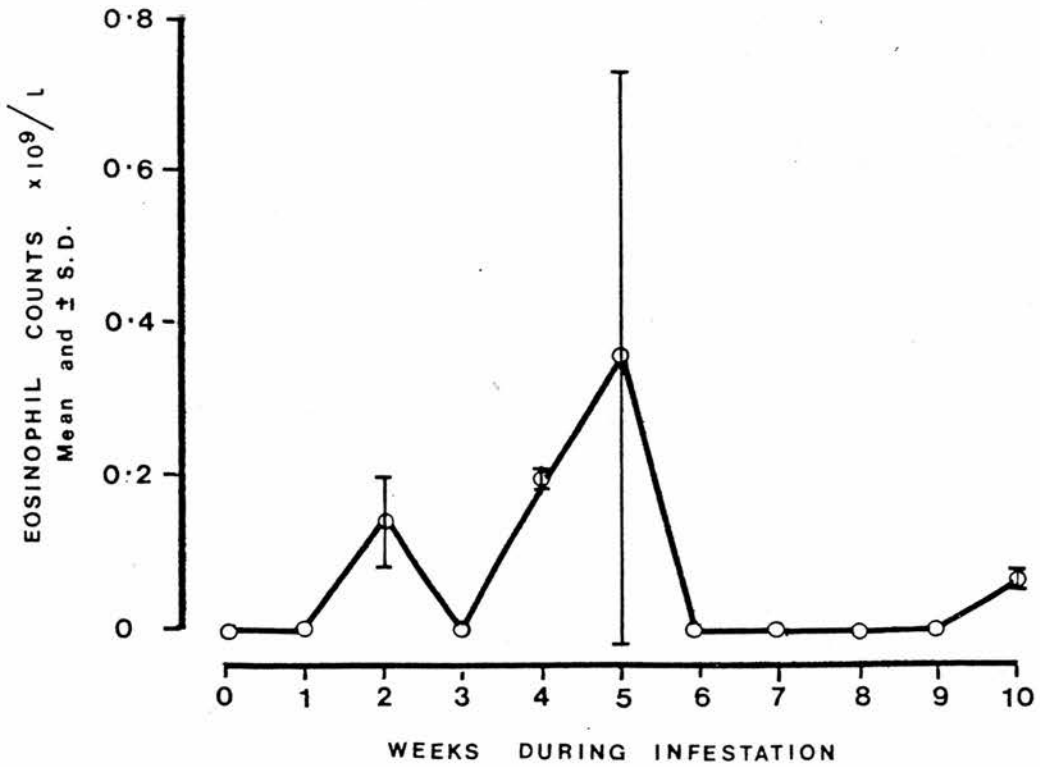


FIG. 4·10



chronic diseases involving continuous degranulation of mast cells. Eosinophilia in response to parasitism occurs when a sensitivity to the protein of the parasite has developed (allergic state) and the protein or secretory product of the parasite is released within the body. Thus they observe that studies of the mechanism of eosinophilia in parasitic infestations indicate that it may be an immune phenomenon. This indication is strongly supported by studies on the effects of repeated tick infestation on the blood composition of the bovine (Hewetson, 1971, van Rensburg, 1959) and on immune responses in the skin of different host species (Cowdry and Dank, 1933; Schleger et al., 1976; Wikel & Allen, 1978). The studies on blood composition revealed eosinophilia in circulation and other workers demonstrated massive tissue infiltration associated with high resistance to tick infestation. In view of these findings it is interesting to observe that no significant elevation in the levels of circulating eosinophils developed in my calves which were exposed to the repeated infestations of R. appendiculatus. However, significant differences were revealed in the eosinophil levels between experimental groups during weeks 0, 1 & 4 but the uninfested group was not involved in the first two analyses because no eosinophils were detected (Table 4.9). The possible explanation for this anomaly is that the immune response to R. appendiculatus was not strong enough to incite

sufficient histamine levels which could induce an eosinophilia (Schalm et al., 1975). Alternatively a possibility of suppressive effect of the stress from chronic exposure can be considered (Schalm et al., 1975). However, the stress could not have been a significant factor during the initial stages of the exposures. Another suggestion would be the tolerance of high histamine levels in the bovine (Herbert, 1970) such that rapid return to base of the elevated levels (Riek, 1955) inhibits manifestation of persistent eosinophilia after tick detachment. Thus there is a possibility that the blood samples were being taken after the peak response of the eosinophils had passed. This could be true for the majority of the estimations, as samples were taken after detachment when the stimulus of engorgement had stopped. In review it can therefore be suggested the low levels of eosinophils recorded in the infested animals was attributable to wrong time of sampling.

4.3.2. Analysis of variance applied to the haematological data revealed significant differences between this uninfested control group and other experimental groups infested with R. appendiculatus in all parameters estimated (Tables 4.1 to 4.9), except neutrophils (Table 4.7). However, the group (I) did not appear in two analyses when significance was revealed because the

TABLE 4.1.

ANALYSIS OF VARIANCE: Significance of Differences between uninfested controls and infested groups of calves in Packed Cell Volumes during regimes of successive infestation of R. appendiculatus instars.

Successive infestation	Groups compared	Degrees of freedom	Variance ratio
0	(I) - (VI)	5,22	0.99
1	-do-	5,22	2.15
2	-do-	5,21	3.08*
3	-do-	5,21	3.72*
4	(I) - (V)	4,19	3.80*
5	-do-	4,18	11.43**
6	-do-	4,18	8.07**
7	-do-	4,15	8.53**
8	-do-	4,15	6.03**
9	-do-	4,13	8.13**
10	(I) & (II)	1,5	1.56

* $P < 0.05 > 0.01$: ** $P < 0.01$

TABLE 4.2.

ANALYSIS OF VARIANCE: Significance of Differences between uninfested controls and infested groups of calves in Red Blood Cell counts during regimes of successive R. appendiculatus instars.

Successive Infestation	Groups Compared	Degrees of freedom	Variance ratio
0	(I) - (VI)	5,22	0.91
1	-do-	5,22	5.91**
2	-do-	5,21	10.79**
3	-do-	5,21	14.03**
4	(I) - (V)	4,19	10.66**
5	-do-	4,18	9.17**
6	-do-	4,18	21.27**
7	-do-	4,15	13.51**
8	-do-	4,15	4.35*
9	-do-	4,13	3.26*
10	(I) & (II)	1,5	2.01

* $P < 0.05 > 0.01$; ** $P < 0.01$

TABLE 4.3.

ANALYSIS OF VARIANCE: Significance of Differences between uninfested controls and infested groups of calves in Haemoglobin Concentrations during regimes of successive infestation of R. appendiculatus instars.

Successive infestation	Groups compared	Degrees of freedom	Variance ratio
0	(I) - (VI)	5,22	0.19
1	-do-	5,22	2.30
2	-do-	5,21	2.90*
3	-do-	5,21	3.73*
4	(I) - (V)	4,19	6.33**
5	-do-	4,18	11.04**
6	-do-	4,18	15.50**
7	-do-	4,15	13.40**
8	-do-	4,15	10.20**
9	-do-	4,13	18.21**
10	(I) \$ (II)	1,5	2.12

* $P < 0.05 > 0.01$; ** $P < 0.01$

TABLE 4.4

ANALYSIS OF VARIANCE: Significance of Differences
between uninfested controls and infested groups of
calves in Mean Corpuscular Volumes during regimes of
successive infestation of R. appendiculatus instars.

Successive infestation	Groups compared	Degrees of freedom	Variance ratio
0	(I) - (VI)	5,22	0.67
1	-do-	5,22	0.99
2	-do-	5,21	1.26
3	-do-	5,21	3.77*
4	(I) - (V)	4,19	6.84**
5	-do-	4,18	3.08*
6	-do-	4,18	4.43*
7	-do-	4,15	4.13*
8	-do-	4,15	2.53
9	-do-	4,13	1.41
10	(I) & (II)	1,5	0.63

* $P < 0.05 > 0.01$; ** $P < 0.01$

eosinophils were not found (Table 4.9). The differences were detected at both 1% (** $p < 0.01$) and 5% (* $p < 0.05 > 0.01$) levels. The highest incidence of the significant differences appeared in the packed cell volumes (PCV), red blood cell counts (RBC) and haemoglobin concentrations (HB) (Tables 4.1 to 4.3) followed by mean corpuscular volumes (MCV) (Table 4.4); total white blood cell counts (WBC), absolute differential counts of lymphocytes and eosinophils (Tables 4.6, 4.8 & 4.9), mean corpuscular haemoglobin concentrations (MCHC) (Table 4.5), in descending order. The differences in PCV, RBC and HB levels were predominantly at the 1% level whereas other erythrocytic parameters were at the 5% level; but the significance for the leucocytic series was all at the 5% level. The high significance in the erythrocytic indices reflects sensitivity which is manifested to a lesser degree in other haematological indices. The demonstration of minimal variance in the mean corpuscular haemoglobin concentrations shows the stability of the index, as the concentration of haemoglobin normally remains fairly constant per cell except under anaemia crises (Schalm *et al.*, 1975). The significant differences in leucocytes appeared in less than 50% of the total estimations made. Comparisons for leucocytic responses cannot be specifically drawn because the studies on blood composition of bovines infested with ticks have not produced significantly consistent results (C.1.2.3). Besides con-

current secondary bacterial infection and/or infestations of other ectoparasites, such as fleas (Larrivee et al., 1964), would invariably influence the circulatory white blood cell responses.

4.3.3. The revealed significant differences between the groups in the different parameters estimated were further analysed by Duncan's New Multiple Range test (DNMRT). The test was applied to the data in which significant variance was detected in order to demonstrate where the differences between the groups lies. The DNMRT resolved the situation by revealing significant subsets in which this uninfested control group (I) responded independently and similarly to the infested groups and also overlapped between subsets (Tables 4.10 to 4.17). The individual subsets comprising one or more groups, designated (I) to (VI) according to the experimental regimes are presented in brackets. The subsets are ranked in numerical ascendancy from the left. The group(s) in each subset are also similarly ranked. Each subset is constituted by groups with closely associated values and in an independent subset the group stands distinctly on its own. The independent subsets involving this group (I) assumed the top end of the ranks in all, except two (Table 4.11, 4.13), the haematological analyses. Further inspection of the subsets will therefore reveal the frequency of concurrence or variance in the numerical ranking between

TABLE 4.5.

ANALYSIS OF VARIANCE: Significance of Differences between uninfested controls and infested groups of calves in Mean Corpuscular Haemoglobin Concentrations during regimes of successive infestation of R. appendiculatus instars

Successive infestation	Groups compared	Degrees of freedom	Variance ratio
0	(I)-(VI)	5,22	3.21*
1	-do-	5,22	0.13
2	-do-	5,21	0.76
3	-do-	5,21	0.50
4	(I)-(V)	4,19	0.14
5	-do-	4,18	0.04
6	-do-	4,18	2.07
7	-do-	4,15	5.65**
8	-do-	4,15	2.35
9	-do-	4,13	0.04
10	(I)&(II)	1,5	0.14

*P < 0.05 > 0.01; ** P < 0.01

TABLE 4.6.

ANALYSIS OF VARIANCE: Significance of Differences between uninfested controls and infested groups of calves in total White Blood Cell counts during regimes of successive infestation of R. appendiculatus instars

Successive Infestation	Groups compared	Degrees of Freedom	Variance ratio
0	(I)-(VI)	5,22	0.97
1	-do-	5,22	2.95*
2	-do-	5,21	0.68
3	-do-	5,21	1.87
4	(I)-(V)	4,19	1.63
5	-do-	4,18	1.44
6	-do-	4,18	4.19*
7	-do-	4,15	3.10*
8	-do-	4,15	2.77
9	-do-	4,13	2.32
10	(I) & (II)	1,5	0.30

* $P < 0.05 > 0.01$; ** $P < 0.01$

TABLE 4.7.

ANALYSIS OF VARIANCE: Significance of Differences
 between uninfested controls and infested groups of calves
 in absolute Neutrophil counts during regimes of successive
 infestation of R. appendiculatus instars

Successive Infestation	Groups Compared	Degrees of freedom	Variance ratio
0	(I)-(VI)	5,22	0.13
1	-do-	5,22	1.95
2	-do-	5,20	0.23
3	-do-	5,21	0.76
4	(I)-(V)	4,19	0.85
5	-do-	4,18	0.73
6	-do-	4,18	2.02
7	-do-	4,15	1.50
8	-do-	4,15	1.36
9	-do-	4,13	1.19
10	(I) & (II)	1,5	1.60

No significant differences $P > 0.05$

TABLE 4.8.

ANALYSIS OF VARIANCE: Significance of Differences between uninfested controls and infested groups of calves in absolute Lymphocyte counts during regimes of successive infestation of R. appendiculatus instars.

Successive infestation	Groups compared	Degrees of freedom	Variance ratio
0	(I) - (VI)	5,22	1.75
1	-do-	5,22	2.73*
2	-do-	5,21	1.01
3	-do-	5,21	2.26
4	(I) - (V)	4,19	3.07*
5	-do-	4,18	1.43
6	-do-	4,18	1.66
7	-do-	4,15	3.13*
8	-do-	4,15	4.07*
9	-do-	4,13	2.08
10	(I) & (II)	1,5	0.02

* $P < 0.05 > 0.01$

TABLE 4.9

ANALYSIS OF VARIANCE: Significance of Differences between uninfested controls and infested groups of calves in absolute Eosinophil counts during regimes of successive infestation of R. appendiculatus instars

Successive infestation	Groups compared	Degrees of freedom	Variance ratio
0	(II) - (V)	3,5	5.45*
1	(II),(III),(V)	2,4	12.37*
2	(I) - (VI)	5,7	1.23
3	.	.	.
4	(I) - (IV)	3,5	5.77*
5	(I) & (II)	1,2	0.11
6	(II) & (IV)	1,2	0.35
7	(IV)	.	.
8	(II) & (IV)	1,4	0.81
9	(II),(IV),(V)	2,4	0.82
10	(I) & (II)	1,2	3.52

* $P < 0.05 > 0.01$. Eosinophils not detected during infestation three and detected only in group (IV) during infestation seven.

the groups in the various indices. Thus the group (I) attained the highest independent ranks in less than 50% of analyses in each of red blood cell counts (RBC), packed cell volumes (PCV), haemoglobin concentrations (HB) and once attained the lowest independent rank in mean corpuscular volumes (MCV) during the sixth infestation (Table 4.13). On the other hand the group appeared in the similar subsets with higher/highest rank at greater than 50% of the analyses in each of PCV, RBC, HB, mean corpuscular haemoglobin concentrations (MCHC), total white blood cell counts (WBC) and absolute differential lymphocyte counts. Conversely, subsets in which the group responded similarly assumed lowest rank at greater than 50% of the analyses in MCV estimations. The rankings revealed by the test demonstrated that the uninfested control group assumed a superior position in the erythrocytic indices. Overall, in the erythrocytic indices the group was associated closest to group (II) (infested with low density of adult instar) followed by groups (V) (infested with larval instar) and (III) (infested with high density of adult instar) and in the leucocytic series the group was closest to group (III) followed by group (II). The associations with groups (II) and (V) were predictable as clinically the calves in these groups withstood the infestations well as will be shown in C.5.1 and C.8.1. Whilst effects were

TABLE 4.10.

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in Packed Cell Volumes during regimes of successive infestation of R. appendiculatus instars.

Successive Infestation	SIGNIFICANT SUBSETS
2	(VI, IV, V, III)(IV, V, III, I, II)
3	(III, VI, IV, V, I)(V, I, II)
4	(III, IV, V, I)(I, II)
5	(III, V)(V, IV)(IV, I)(I, II)
6	(III, V)(V, IV)(IV, II, I)
7	(III, V, IV)(V, IV, II)(II, I)
8	(III, IV, V, II)(II, I)
9	(IV, III, V)(III, V, II)(I)

TABLE 4.11.

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in Red Blood Cell counts during regimes of successive infestation of R. appendiculatus instars.

Successive Infestation	SIGNIFICANCE	SUBSETS
1	(VI, III, V, IV)	(II, I)
2	(VI, III, IV, V)	(I, II)
3	(III, VI, IV, V)	(I) (II)
4	(III, IV, V, I)	(I, II)
5	(III, V, IV)	(V, IV, II) (I)
6	(III, V)	(V, IV, II) (I)
7	(III, V, II, IV)	(I)
8	(III, V, IV, II)	(II, I)
9	(III, IV, V, II)	(I)

TABLE 4.12

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in Haemoglobin Concentrations during regimes of successive infestation of R. appendiculatus instars.

Successive Infestation	SIGNIFICANT SUBSETS
2	(VI, IV, III, V)(IV, III, V, I, II)
3	(VI, III, IV, V, I)(V, I, II)
4	(III, IV, V)(I, II)
5	(III)(IV, V)(I, II)
6	(III)(V, IV)(II, I)
7	(III, V, IV)(V, IV, II)(I)
8	(III, IV, V, II)(I)
9	(IV, III)(III, V)(V, II)(I)

TABLE 4.13

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in Mean Corpuscular Volumes during regimes of successive infestation of R. appendiculatus instars

3	(I, II, VI)(II, VI, IV)(VI, IV, III, V)
4	(II, I)(I, V)(V, III)(III, IV)
5	(I, III)(III, V, IV, II)
6	(I)(III, IV, V, II)
7	(I, III)(III, IV, V, II)

TABLE 4.14

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in Mean Corpuscular Haemoglobin Concentrations during regimes of successive infestation of R. appendiculatus instars.

Successive Infestation	SIGNIFICANT SUBSETS
0	(VI,I,III,II,V)(II,V,IV)
7	(II)(IV,V,II,I)

TABLE 4.15

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in total White Blood Cell counts during regimes of successive infestation of R. appendiculatus instars.

1	(VI,IV,V,III,I)(V,III,I,II)
6	(IV,V,II)(I,III)
7	(V,IV,II,III)(II,III,I)

TABLE 4.16.

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in Absolute Lymphocyte counts during regimes of successive infestation of R. appendiculatus instar.

1	(IV,VI,V,I,III)(V,I,III,II)
4	(IV,V)(V,III,I,II)
7	(V,IV,II,III)(II,III,I)
8	(IV,V,III)(V,III,I,II)

TABLE 4.17

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in absolute Eosinophil counts during regimes of successive infestation of R. appendiculatus instars.

Successive Infestation	SIGNIFICANT SUBSETS
0	(III, IV, V) (II)
1	(V, II, III)
4	(IV, III, I) (III, I, II)

serious during the individual infestations with low density adult of Rhipicephalus appendiculatus the interval between exposures allowed adequate recovery so that the haematological responses were not severely depressed.

4.3.4. Biochemical estimations.

The mean values and standard deviations of serum levels of total protein (S.P.), immunoglobulins (IG) and glucose (S.G.) are presented in Figures 4.11 to 4.13. The levels were within the normal ranges (Coles, 1974; Irwin, 1974) except ^{for} the initial low values in the immunoglobulin estimations. The low mean values of IG were partly attributable to poor levels shown by the two calves (p.130) and this anomaly could have predisposed them to the nonspecific clinical disturbances they evinced. The depressed levels in the three parameters coincided with the period of unsettled temperature reaction (Fig. 4.1) after which higher levels obtained. The mechanism underlying the depression is not clear. The individuals showed fairly consistent small variation per week illustrated by equally small standard deviations. The missing values for serum glucose were due to a limited supply of reagents. It is noteworthy that the responses in the serum protein and immunoglobulins showed close similarity from week two onward (Figs. 4.11 to 4.12). The decline of serum protein initially could be associated

FIG. 4.11

Means and standard deviations of Serum Protein levels of five uninfested control calves during experimental periods.

FIG 4.12

Means and standard deviations of Immuno-globulins levels of five uninfested control calves during experimental periods.

FIG. 4·11

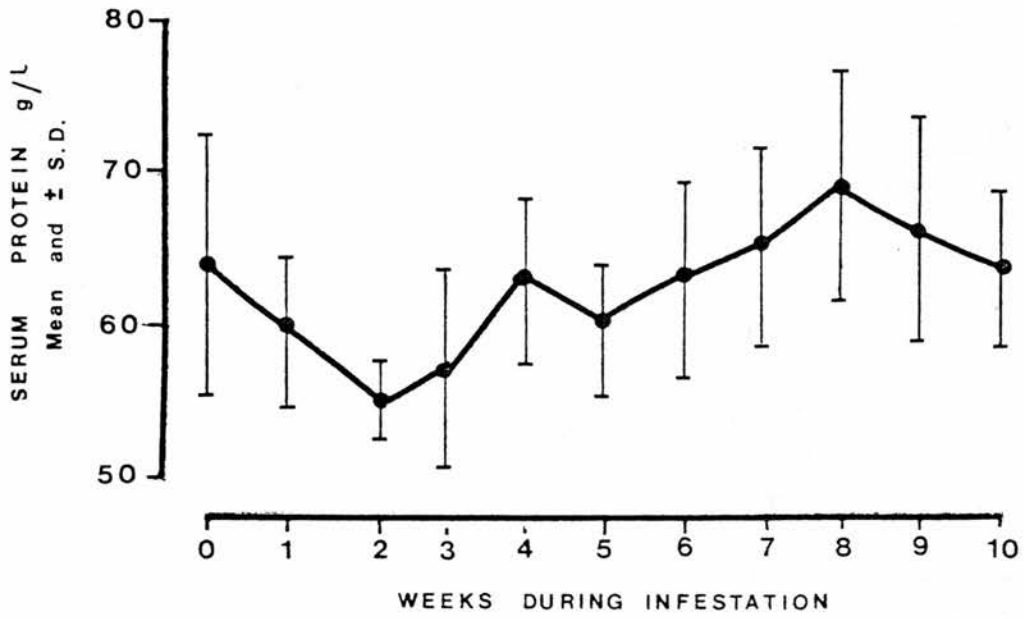
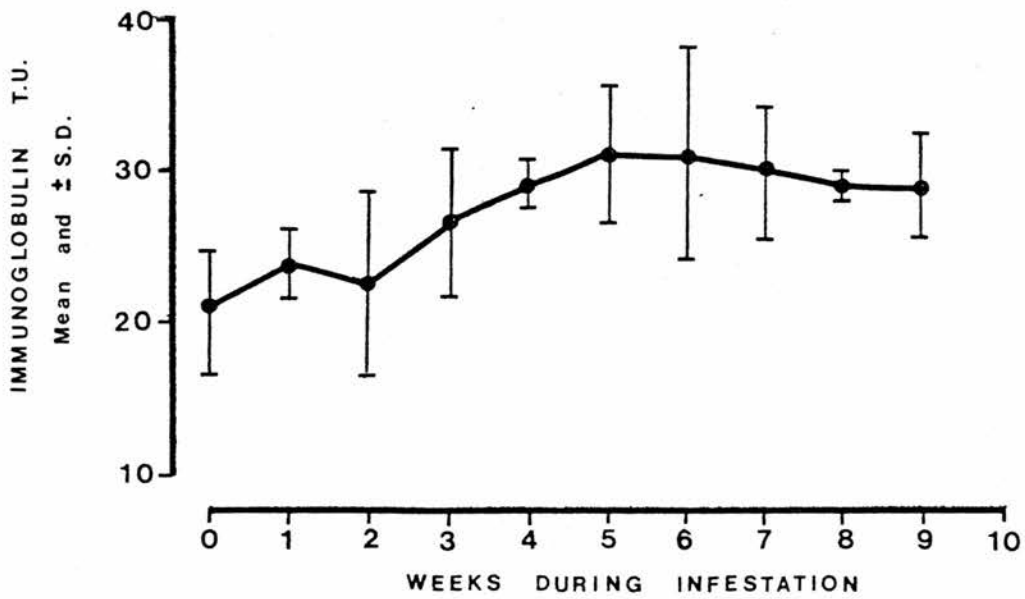


FIG. 4·12



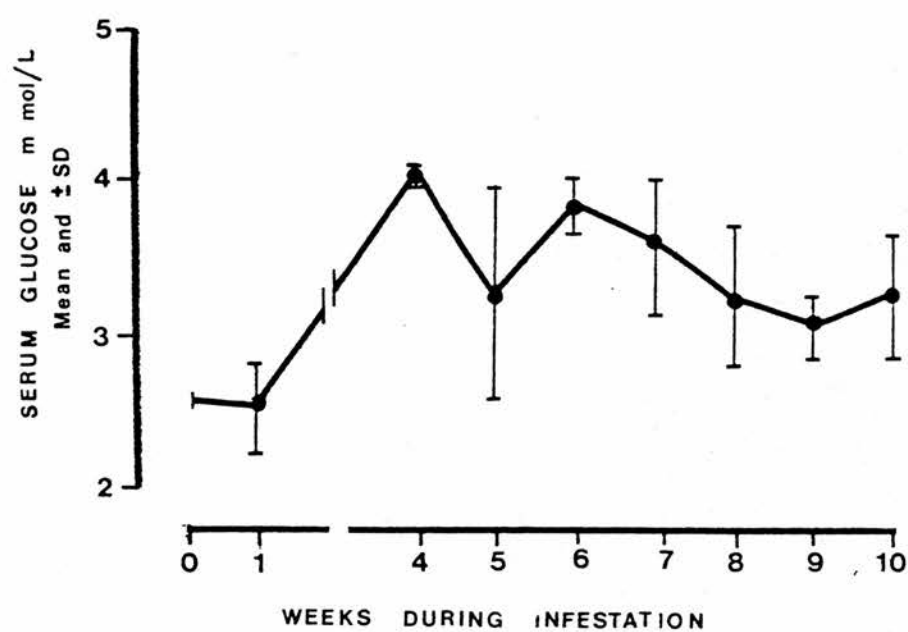
with pyrexia-diarrhoea reactions which can interfere with protein metabolism. The diarrhoea would cause malabsorption but the influence of pyrexia in these calves which were in good body condition remains obscure. The subsequent rise in the levels could be attributed to feeding predominantly and, in addition, to globulin synthesis stimulated by the non specific infections. Moreover, the pyrexia and diarrhoea were almost settled by then. The data for serum albumin, a more sensitive index to tick effects (O'Kelly & Seifert, 1970) were not used because of being incomplete as explained before (C.2.6.2).

The mean values of serum glucose were high though low levels obtained initially (Fig. 4.13). Comparison of serum glucose levels of this uninfested control group and other groups exposed to R. appendiculatus is limited by the incomplete data caused by missing figures. Nonetheless, as shown in Tables 4.20 and 4.23, the highly significant differences involving the group appeared once during estimations for week one. However, the mean values for calves in group (III) (infested with high density adult instar) were slightly lower (Fig. 6.16). It should be pointed out, however, that the serum glucose is rather an insensitive index in cattle (Coles, 1974; Medway et al., 1969) and again reports on serum levels from studies of blood composition in tick infested bovines are scarce (O'Kelly et al., 1971). Thus the serum glucose was an unsuitable index for testing specific tick effects on host metabolism.

FIG 4.13

Means and standard deviations of Serum
Glucose levels of five uninfested control calves
during experimental periods.

FIG. 4.13



4.3.5. Analysis of variance revealed significant differences between the uninfested control group and other experimental groups in the three parameters. The differences revealed equally at 1% and 5% levels were infrequent and confined to the estimations in the first half of infestations (Tables 4.18 to 4.20). However, differences for serum glucose during the ninth analysis just failed to attain significance because of low degrees of freedom (Table 4.20). Thus the serum glucose (S.G.) showed little significant variance between all groups tested. It is therefore appropriate to suggest that the parameters were generally not sensitive indicators for specific effects caused by repeated infestations of Rhipicephalus appendiculatus instars.

Duncan's New Multiple Range test, applied as before, resolved the differences by indicating subsets in which the uninfested control group (I) responded independently and similarly and also overlapped between subsets (Tables 4.21 to 4.23). The group appeared in subsets with least values on four occasions and twice ranked lowest during exposures in weeks 1, 2, 3, 4; and 1 & 2, respectively (Tables 4.21 & 4.23). Paradoxically absence of significant differences in the immunoglobulins during infestation (Table 4.19) indicated poor immunological response to tick feeding. It is, however, highly unlikely^{that}/all regimes equally inhibited the immunoglobulin producing system to produce identical levels as obtained in the control group. This lack of significant differences between groups in

immunoglobulin levels cannot be specifically explained.

The test associated the control group, during exposures, most closely with experimental groups (III) and (VI) (infested with high density adult instar and concurrent three instars of Rhipicephalus appendiculatus respectively), followed by groups (IV) and (V) (nymphal and larval infestations respectively) and least with group (II) (low density adult instar infestation). The closest association with groups (III) and (VI), which sustained the most severe effects from infestations, coincided with the obscure initial decline in serum protein in this group (I) (Fig. 4.11).

4.4. AUTOPSIES

Since all calves lived through the experiments, then there were no autopsies performed. Slaughter to compare responses to tick effects was not done either. This was not practicable because rearing of calves obtained as neonates proved difficult. Therefore there was need to conserve the uninfested control calves.

4.5. SUMMARY OF SIGNIFICANT FINDINGS

Clinically the calves in the uninfested control group (I) maintained quite good health, keen appetite for the feeds given and active physique. However, mild transient clinical manifestations, e.g. pyrexia, diarrhoea and coughing, were evinced due to non-specific

TABLE 4.18.

ANALYSIS OF VARIANCE: Significance of Differences between uninfested controls and infested groups of calves in Serum Protein levels during regimes of successive infestations of R. appendiculatus instars.

Successive Infestation	Groups compared	Degree of freedom	Variance ratio
0	(I) - (VI)	5,22	6.50**
1	-do-	5,22	1.08
2	-do-	5,21	3.20*
3	-do-	5,21	2.83*
4	(I) - (V)	4,19	3.66*
5	-do-	4,18	2.41
6	-do-	4,18	2.48
7	-do-	4,15	1.31
8	-do-	4,15	3.00
9	-do-	4,13	1.92
10	(I) - (II)	1,5	0.01

* $P < 0.05 > 0.01$; ** $P < 0.01$

TABLE 4.19.

ANALYSIS OF VARIANCE: Significance of Differences
between uninfested controls and infested groups of calves
in Immunoglobulin levels during regimes of successive
infestation of R. appendiculatus instars.

Successive Infestation	Groups compared	Degree of freedom	Variance ratio
0	(I) - (VI)	5,18	6.47**
1	(I) - (V)	4,17	1.85
2	-do-	4,15	1.99
3	-do-	4,16	0.88
4	-do-	4,14	1.27
5	-do-	4,13	0.32
6	(I)-(II)-(V)	2,9	1.12
7	(I) - (V)	4,10	1.29
8	(I) - (IV)	3,9	1.93
9	(I),(II),(IV),(V)	3,8	1.43

* $P < 0.05 > 0.01$; ** $P < 0.01$

TABLE 4.20.

ANALYSIS OF VARIANCE: Significance of Differences
between uninfested controls and infested groups of calves
in Serum Glucose during regimes of successive infestation
of R. appendiculatus instars.

Successive infestation	Groups compared	Degrees of freedom	Variance ratio
0	(III),(IV),(V),(VI)	3,10	4.51*
1	(I)-(V)	4,14	6.15**
2	(II)-(V)	3,11	10.51**
3	-do-	3,11	1.43
4	(I)-(V)	4,12	2.81
5	-do-	4,10	1.03
6	(I),(II),(III),(V)	3,8	2.92
7	(I),(II),(V)	2,6	1.67
8	(I),(II)	1,5	0.76
9	-do-	1,5	5.97
10	-do-	1,3	3.42

* $P < 0.05 > 0.01$; ** $P < 0.01$.

TABLE 4.21

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in Serum Protein Levels during regimes of successive infestation of R. appendiculatus instars.

Successive infestation	SIGNIFICANT SUBSETS
0	(II, V, I)(V, I, III, IV)(III, IV, VI)
2	(I,VI, V)(VI,V,III,II)(V,III,II,IV)
3	(VI, I, III, IV, V)(IV, V, II)
4	(III, I, V, IV)(V, IV, II)

TABLE 4.22

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested group of calves in Immunoglobulin levels during the pre-infestation period.

0	(I),(II,VI, IV, III)(VI, IV, III, V)
---	--------------------------------------

TABLE 4.23

DUNCAN'S NEW MULTIPLE RANGE TEST: Significant Subsets derived from Significant Variance between uninfested controls and infested groups of calves in Serum Glucose levels during regimes of successive infestation of R. appendiculatus.

0	(VI, IV, III)(V)
1	(I,VI,IV,II)(VI,IV,II,V,III)
2	(III,IV)(IV,V)(II)

causes.

Clinical-pathological estimations produced mean values within normal ranges, but the leucocytic series showed some slight elevations. Overall the standard deviations were small. The absolute values for eosinophils were quite low in all groups despite repeated infestation with R. appendiculatus instars. Significant statistical differences were revealed between the uninfested control group and the infested experimental groups in the haematological and biochemical estimations. The variance appeared in more than 50% of the analyses for the erythrocytic indices and in less than 50% for the leucocytic series and biochemical parameters. The infrequent variance in the biochemical analyses was confined to the first half of the exposures. The indices which showed significant differences during exposure were packed cell volumes, red blood cell counts, haemoglobin concentrations, mean corpuscular volumes, mean corpuscular haemoglobin concentrations; total white blood cell counts, absolute differential counts of lymphocytes and eosinophils; serum protein and serum glucose. The reason for lack of significant differences between groups in the immunoglobulin levels is not clear. Thus the biochemical parameters were in general less sensitive to effects of tick infestations than the haematological findings.

The uninfested control group definitely contrasted with the infested groups in clinical and haematological

evaluations. Nonetheless, the group (I) showed closest association with group (II) (infested with low density adult instar) followed by group (V) (infested with larval instar) in the overall responses in the erythrocytic indices. This association was predictable because these groups sustained the least systemic disturbance from exposure to R. appendiculatus instars.

CHAPTER 5

EXPERIMENT (II): RESPONSES OF FIVE CALVES EXPOSED
FORTNIGHTLY TO SUCCESSIVE LOW DENSITY INFESTATIONS OF
ADULT RHIPICEPHALUS APPENDICULATUS.

The calves aged three to eight months and previously unexposed to ticks were clinically healthy at the first tick application. The mean numbers of the ticks applied and engorged females recovered during successive infestations are presented in Fig. 3.1. The reactions of the calves to the infestations revealed by physical examination and blood composition estimations were as follows.

5.1. PHYSICAL (CLINICAL) SIGNS

5.1.1. It was determined, by observation and physical examination of the individual systems, that the responses induced were as follows. The immediate response to attachment, especially during secondary infestations, was associated with pain and irritation. The common signs observed were frantic struggling and shaking of the head and ears, restlessness, increased ocular/nasal/salivary secretions and frequent urination, and often the animals stopped eating for some hours. The manifestations observed subsequently are described on a system basis following the format used at our clinics.

5.1.2. Generally the loss in body condition was slight at first but became moderately severe after four to five infestations. However, the condition of one calf (number T.5) deteriorated much earlier due to pulmonary complications. Its condition declined rapidly and ^{it} died during the fifth infestation, having sustained an irreversible cachexia which was confounded by inappetence. Another calf (number T.3) became extremely wasted and showed ^a small pendulant abdomen during the eighth to tenth infestations. Ballotment of the abdomen gave signs suspicious of ascites (accumulation of fluid in peritoneal cavity). Centesis was not carried out to confirm this suspicion.

The calves initially showed bright and active de-meanour and physique but weakened in the last three infestations as a result of prolonged exposure. However, two of the calves which developed respiratory complications were depressed much earlier and one of them (T.5) became severely depressed and weakened during the last two infestations before death. Stress caused by fever, coughing and mild diarrhoea had a major influence in accelerating the decline in the condition.

5.1.3. The cardiovascular system being quite sensitive, was investigated in much greater detail than other systems. Thus the parameters recorded included the following. Pyrexia was a prominent clinical sign. All calves showed slight to severe pyrexia during

FIG. 5.1

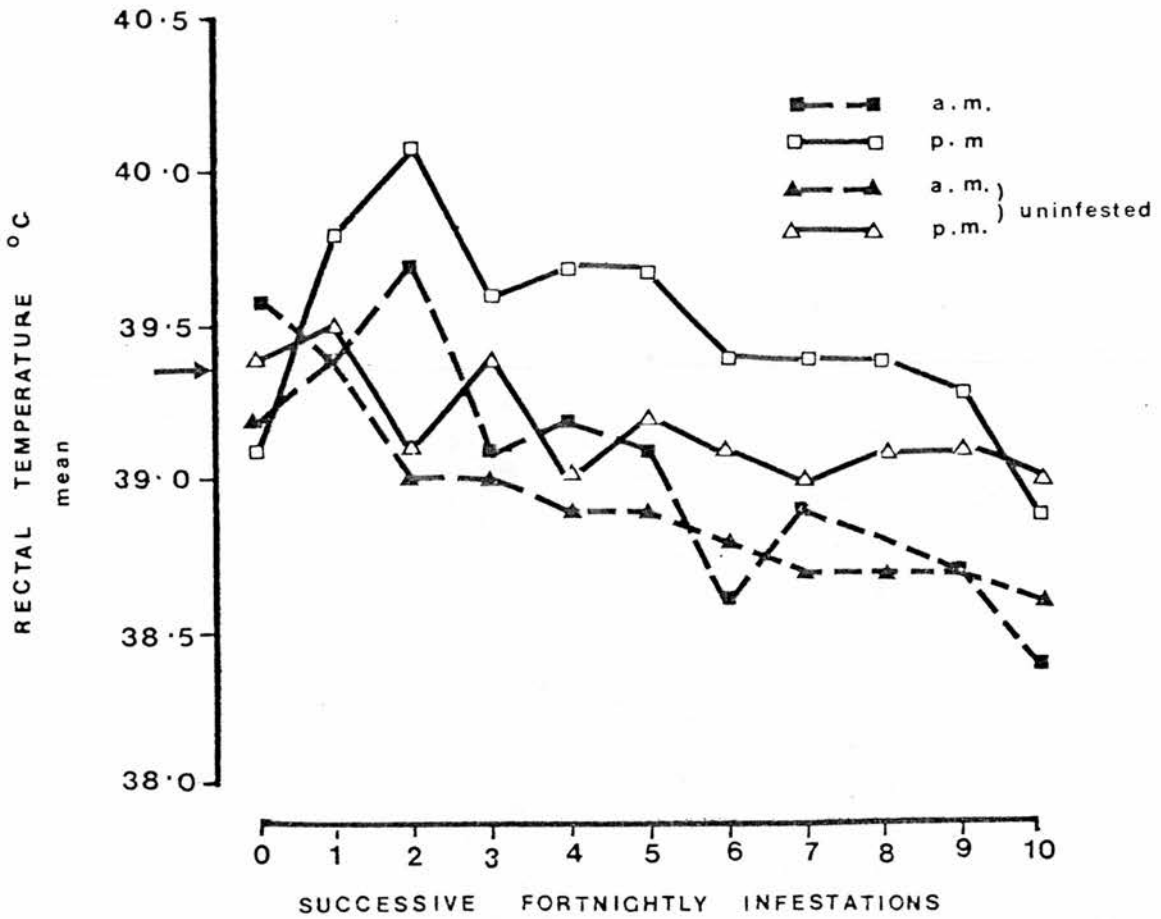


FIG. 5.1.

Mean weekly Rectal Temperatures of five calves exposed fortnightly to successive low density infestations of adult Rhipicephalus appendiculatus compared with uninfested controls. (Arrow marks normal critical temperature). (n) reduced to four calves after 6th infestation.

infestations. The evaluation of the pyrexia was based on this scale: 39.3°C = critical normal level; $39.3^{\circ} + 0.1^{\circ}$ to 0.5° = (+) slight; $+ 0.6^{\circ}$ to 1.0° = (++) moderate; $+ 1.1^{\circ}$ to 2.0° = (+++) marked; and $+ 2.1^{\circ}$ and more = (++++) severe. The mean rectal temperatures for the seven-day period during the successive infestations are presented in Fig. 5.1. This length of time was adopted because in most cases the female ticks completed feeding within seven days after application. Thus the degree of mean pyrexia was slight to moderate but finally showed a stepwise decline to almost normal temperature. The pyrexia recorded during the pre-infestation period (a.m.) was of non-specific origin as a similar reaction occurred in the uninfested control group as shown in Fig. 5.1. The lowest pyrexia levels coincided with reduced tick burden (Fig. 3.1). The trend in the levels of the pyrexia showed a significant regression to normal rectal temperature ($r = - 0.86$, a.m. & $- 0.89$, p.m.; $p < 0.001$). The trend in the mean weekly temperatures indicates an inhibition of the pyrogenic activity of tick secretions during latter infestations. The results in Fig. 5.1 at three points, viz : first, second and seventh successive infestations, are further amplified as illustrated in Figures 5.2 to 5.4 . The amplification illustrates the mean daily rectal temperatures. Figure 5.2 shows the mean daily rectal temperature during the first infestation.

FIG. 5.2

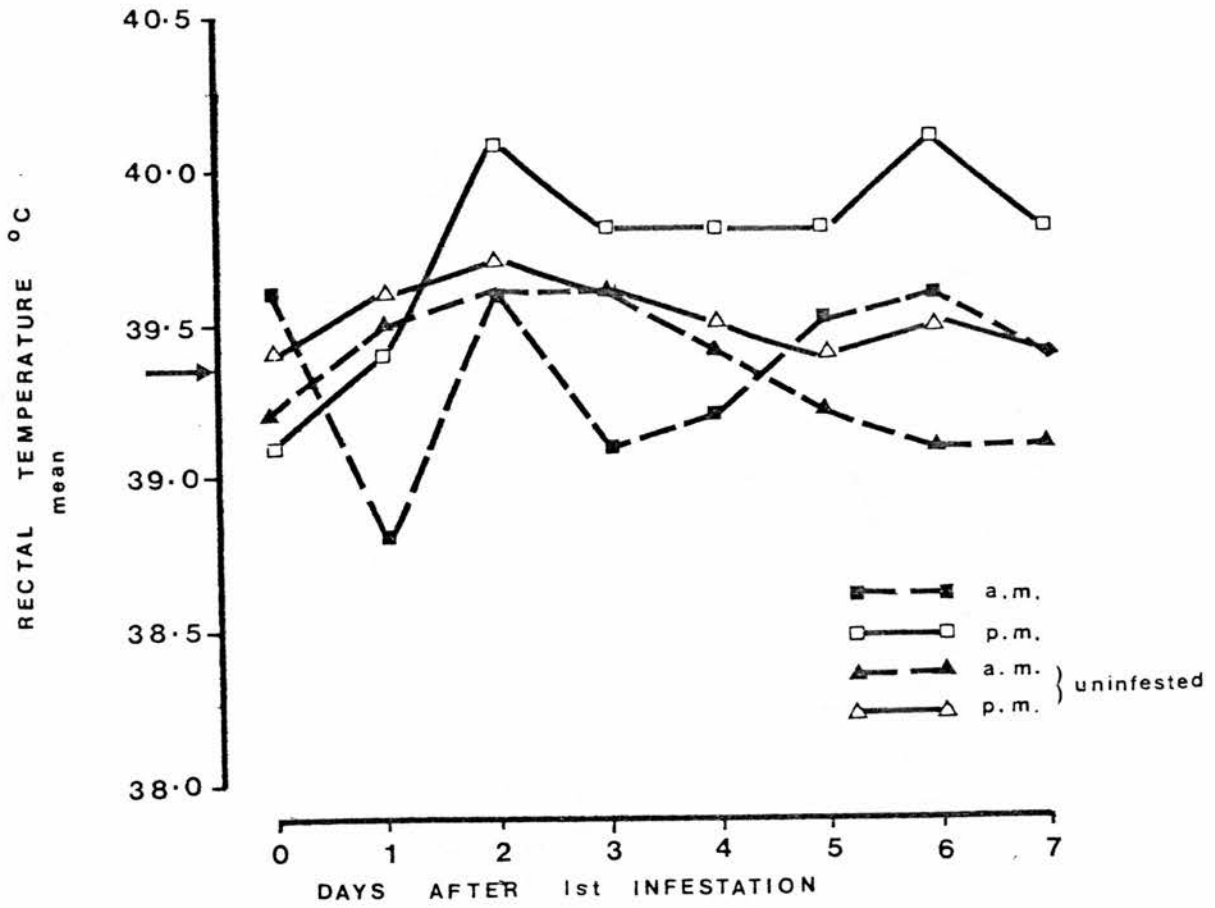


FIG. 5.2

Mean daily Rectal Temperatures of five calves exposed to first low density infestation of adult Rhipicephalus appendiculatus compared with uninfested controls. (Arrow marks critical normal temperature).

Contrary to expectation two pyrexia peaks developed and the first rise did not conform to the very active feeding phase associated with engorgement of female ticks responsible for the second rise. Most probably the early pyrexia followed by a remission was non-specific as it also appeared in the uninfested control group. In contrast, Fig. 5.3 illustrates the response to the second successive infestation and, as expected, a diphasic reaction developed. The development of the pyrexia levels was associated with both the initial phase of feeding and engorgement of the female ticks. Thus the initial pyrexia level indicated that the animals reacted through^a/sensitization mechanism to the tick secretion(s) (Aitkin, 1960). Figure 5.4 illustrates a reversal in the pyrexia picture during the seventh successive infestation. It is noteworthy that the pyrexia occurred early and then disappeared when the females were engorging. This would suggest that the number of engorging female ticks was not high enough to induce the pyrexia. However, similarity between the numbers of engorged females recovered then and at the fifth infestation (Fig. 3.1) with much higher pyrexia level (Fig. 5.1) would discount the suggestion. It can be further suggested that the host system became tolerant to the pyrogenic stimulus of the tick secretions as a result of repeated infestations. The erratic pyrexia recorded in the uninfested control group during the first three weeks (Fig. 5.1 to 5.3) was of non-specific

FIG. 5.3

Mean daily Rectal Temperatures of five calves exposed to second successive fortnightly infestation of low density adult Rhipicephalus appendiculatus compared with uninfested controls. (Arrow marks critical normal temperature)

FIG. 5.4

Mean daily Rectal Temperatures of five calves exposed to seventh successive fortnightly infestation of low density adult Rhipicephalus appendiculatus compared with uninfested controls. (Arrow marks critical normal temperature). (n) was four calves.

FIG. 5.3

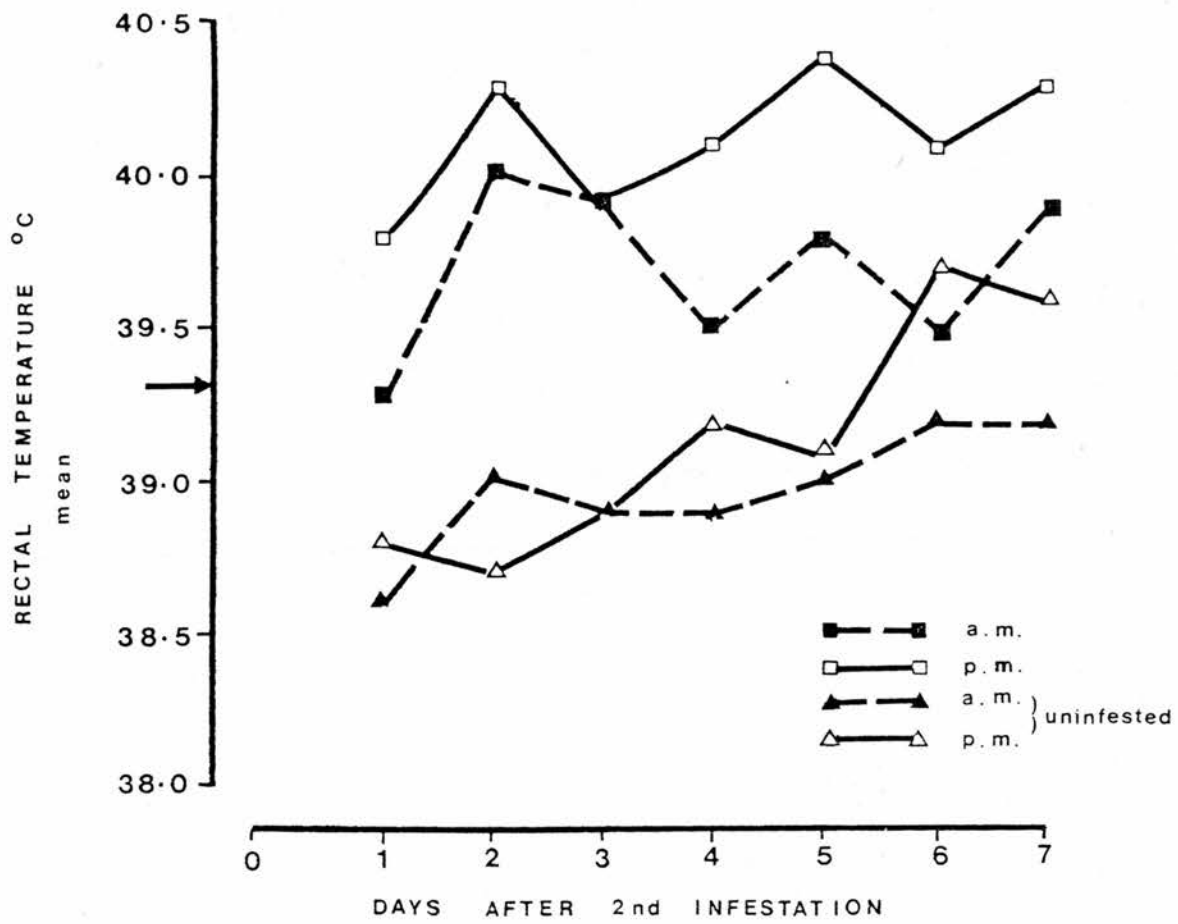
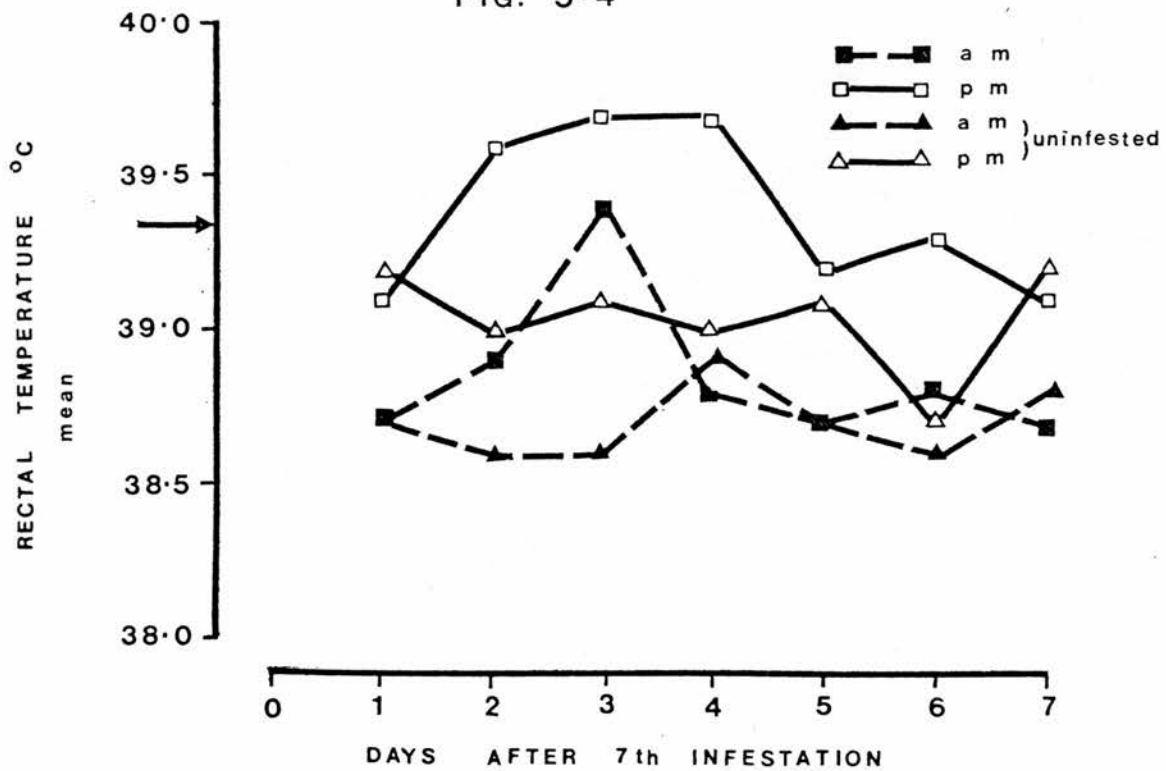


FIG. 5.4



origin. Heart and pulse activity showed no long lasting alterations. Nevertheless, temporary elevations (102 to 156/minute) were found in different calves at various times and were mostly associated with pyrexia and severe reaction of the ear(s) which manifested swelling, oedema and pain. There was a concurrent increase in force of the heart beats and pulse wave but the rhythm remained regular. Erythema and oedema developed around the infested areas. Initially erythematous spots formed round bite wounds but these extended to cover the entire pinna as tick feeding continued. The oedema developed in the same manner. During secondary infestations the reactions were more intense and developed more rapidly, accompanied by heat and pain as detected by palpation of the affected sites. Thus affected calves loathed handling of the reacting tissues. The severity of the reactions was influenced by the density of ticks applied, i.e. the higher density caused severer effects. Whereas hypersensitivity, with bleb formation (described later), caused a severe reaction the resultant inhibitory effect on tick feeding itself shortened the course of the responses. Severe localised oedema made the ears droopy and occasionally involved other sites in the contiguous areas, such as the eyelids and subparotid regions. The distribution of erythema showed a similar pattern and

in repeated infestations was seen in unpigmented skin remote from attachment sites. The most intense response was found in three out of the five calves and in all cases subsided rapidly after the engorged female ticks had detached. The generalised erythema indicated that sensitization developed in areas beyond infested sites and that the tick secretions got into circulation rapidly. Mucous membranes and secretions (discharges) presented these features. The visible mucosae remained generally normal (i.e. pink, moist, glistening and intact) though in the majority of the infestations the conjunctivae on the infested side(s) became congested to varied degrees. This localised reaction shows that the tick secretions have a direct effect on the circulatory activity within the vicinity of the infested sites. These inflammatory changes are similar to findings made by Thomas & Neitz (1958) who demonstrated serosanguineous fluid from the subcutis of infested areas. Excessive lachrymation from the affected eye(s) developed and occasionally the secretion became mucopurulent. Two calves had nasal discharge for several days per infestation but it was associated with chronic pneumonia in one (T.5). However, for no apparent reason the second calf also secreted copious frothy saliva for several days, especially at night, during the seventh infestation. The saliva collected in the feed trough made of metal. Examination of the mouth revealed no visible lesion which could be responsible for the excessive salivation.

5.1.4. The lymphatic system showed a regular and significant response. All calves evinced varied lymphadenopathy in the regional drainage nodes. The first discernible increase in size generally occurred round the third day post infestation and persisted for several days after all ticks had detached. The lymph node(s) were just palpable one month later in the absence of further challenge. The parotid nodes attained maximum size three to five times the pre-infestation measurement. However, persistent enlargement occurred in one calf (T.5) after the second successive infestation and at autopsy, nearly two months later, an abscess was found. This was confounded by otitis externa during the fourth infestation. Lymphadenopathy became less marked after the fourth successive infestation. The subdued response was most noticeable with severe bleb formation (in two calves) and in general coincided with reduced numbers of successfully engorging female ticks (Fig. 3.1). Thus it can be suggested that the subdued reaction was influenced by the reduced source of stimulus of the engorged female ticks. It is possible that the necrotic changes in the lymphoid follicles observed by van Rensburg (1959) could inhibit further enlargement of the nodes.

5.1.5. The respiratory system showed a moderate increase in the respiratory rate (36 to 48/minute), and coughing in all animals at different times for varied periods. The coughing was at least partly due

to non-specific causes as it concurrently affected the uninfested control group. Mucoid and mucopurulent nasal discharges appeared in two calves and were observed both with and without spontaneous coughing. However, one calf with acute pneumonia became severely dyspnoeic (i.e. rate 72/minute, expiratory grunt, extended neck, oral breathing and frothing) in the terminal stages. The chronic pneumonia defied antibiotic treatment and bacteriological investigation at autopsy revealed a pure culture of Corynebacterium pyogenes organisms obtained from abscessed lymph node and lung tissues. Clinical diagnosis of the causative agent was not possible since the lymph node association was not suspected. Moreover, the persistently enlarged node was not explored by centesis nor did spontaneous bursting of the abscess occur. The link between the node and the lungs is through the efferent lymphatic vessels, thoracic duct and the right heart. The vulnerability of the pulmonary tissue to infection is attributable to the position of the lungs in respect to the circulatory system whereby it filters and diffuses the whole circulatory volume (Jubb & Kennedy, 1970). However, the autopsy findings on the dead calf revealed a suppurative bronchopneumonia (see C.5.3.). The dominant gross lesions in this calf appeared in the apical lobes with an extension to one diaphragmatic lobe. This conformed to the development of airborne pneumonias as described by Pirie (1979). He suggested that the involvement of the anterior segments of the lungs in natural infections may be influenced by other important factors. Such factors can be associated with

the less efficient defense mechanisms in the segments for removing inhaled material. It is possible the defensive mechanisms of this calf were affected by the stress from tick feeding which was compounded by the permanent housing and this would agree with Pirie's (1979) observations on the incidence of pneumonia in calves kept in confined quarters. The incrimination of the aerogenous mode of infection is strongly supported by the fact that the uninfested control calves sustained protracted coughing and pyrexia during the experiments though no deaths occurred. If the pneumonia was haematogenous in origin then the gross lesions would show a different distribution which would conform to the vulnerability of the pulmonary tissues described by Jubb & Kennedy (1970). Pirie (1979) observed that the lesions caused by the haematogenous infection tend to be distributed in the diaphragmatic lobes, although all the lobes are affected when a large number of organisms are present. According to the description of Jubb & Kennedy (1970) the cytotoxic effects of the invading organisms and the transfer of parasitized cells would predispose the tissues to serious inflammatory reactions. Unfortunately the lack of histological evidence in my study prevents a precise definition of the distribution of the lung lesions. Nonetheless, it would be reasonable to suggest that the bronchopneumonia was exacerbated by the organisms released from the lymph node abscess and the chronic effects of tick feeding.

Thus the cytotoxic effects of the invading organisms and the transfer of parasitized cells would predispose the tissues to serious inflammatory reactions.

Corynebacterium pyogenes is a pyogenic organism and produces a potent necrotising toxin which can lead to severe tissue necrosis and abscessation. The proliferation of the organisms is facilitated by high oxygen tension in the lungs since C. pyogenes is an aerobe. The encapsulation and thick pus constituting the lesions create a barrier to effective diffusion of the drugs administered, hence the intractable nature of the pneumonia. It is possible that this clinical combination, lymph node abscess-bronchopneumonia, is more frequent under field conditions than has occurred in this study. The autopsy findings in C.6.3 and C.9.3, where calves with abscessed lymph nodes were withdrawn and slaughtered, give strong indications of high incidence. In support of this observation several workers have reported pyogenic infections locally and in deep seated tissues associated with infestations of R. appendiculatus and other species of ticks. Thomas & Neitz (1958) and van Rensburg (1959) observed many cases of lymph node abscessation during their studies on rhipicephaline toxicosis. The abscesses burst spontaneously or were found at slaughter but no related pulmonary changes were reported.

Macleod (1933), Wood (1968), Hoogstraal (1956) Clifford (1954) describe secondary infections locally and in drainage lymph nodes which may culminate in pyaemia. Foggie (1959) described the relationship of tick bite to tick pyaemia in lambs. The pyaemia in

young animals is commonly associated with lung abscesses and joint infection. du Toit & Theiler (1964) stated that tick bite wounds facilitated severe secondary local and deep seated tissue complications, e.g. suppuration and abscessation, which end in death or deformities. The inflammatory events which can lead to secondary infection of drainage lymph nodes and subsequently other tissues, including the lungs, are illustrated by Theis et al., (1976) in their study on the changes in lymphatic fluid draining an area infested by R. sanguineus on the dog. They commented on the flowing lymph acting as a vehicle for transmissible infections. Thus the chances of spread of the common pyogenic organisms on the skin, e.g. Corynebacterium spp., to cause secondary infection (abscesses-pneumonia) would be increased by the severe local inflammatory reaction produced in cattle heavily infested with R. appendiculatus.

5.1.6. Digestive system function showed generally keen appetite but the keenness waned temporarily for varied periods in four out of the five calves. Diarrhoea, generally mild, occurred on several occasions and at times coincided with the severe hypersensitivity state (clinical reaction); nevertheless, the cause could have been non-specific as the uninfested control group suffered concurrently.

The urinary system showed no gross abnormalities except frequent urination associated with immediate

the reaction to/attachment and engorgement phase of the applied ticks. This seems to be a reflex stimulus related to pain sensation. The musculo-skeletal system showed deterioration in function and conformation associated with decline in general body condition and physique. Thus the calves became thin and weak but did not progress to prostration.

5.1.7. Nervous system observations were predominantly associated with pain and irritation sensations. Tick feeding, especially in secondary infestations, induced much pain and irritation which caused restlessness and pruritis. Other nervous signs have been described under immediate responses (C.5.1.1). Infested ears became markedly tender due to oedematous swelling and bleb formation which made the animals resist handling. The pain sensation could be attributed to the effect of possible accumulation of histamine and tension on pain nerve-endings as the skin stretches due to the oedema. Pruritis of varying intensity, also related to histamine release, was induced constantly in all calves and was manifested by vigorous attempts to groom. The grooming behaviour involved rubbing against objects, pawing with hind feet (scratching) supplemented by vigorous head/ear shaking. Where restraint was very restrictive the animals stamped and made jerky movements as if trying to break loose. The urge to groom abated when all female ticks had completed feeding and detached. However, stray females in small numbers at sites away

from the ears did not cause appreciable irritation. This could partly be attributed to such sites being less accessible to grooming because of restraint. The feeding of male ticks alone caused hardly any irritation. This is supported by observations of big clusters, especially over ears, which remained undisturbed for several weeks after all the females had detached and the animals unrestrained. Where grooming succeeded the infested areas became markedly abraded.

5.1.8. The integumentary system was another system investigated closely because of the accessibility in examination and the vast amount of information recorded by other workers. The direct reactions were associated with plain bite wounds and primary inflammatory responses, exudation from wounds followed by encrustation, scarring and distortion, transformation of the epidermal layer (pityriasis and hyperkeratosis), and abrasions of pruritic origin. The exudate and encrustations caused "scalding" and depilation but the latter reaction also developed in the absence of severe epidermal disturbance. In addition bite wounds bled much, especially on detachment of engorged ticks, and were accompanied by a reaction in the surrounding tissue. Resolution occurred rapidly in the absence of complications, under scabs which exposed scars and baldness on lifting. The baldness was temporary as hair regrowth occurred in about four weeks and the scars could not be recognised after some weeks. Dense clustering induced severe reaction leading to

distortion of the aural cartilage on healing. The pinna curled at the tip or the edges and surfaces appeared wrinkled. The distortion could be attributed to contraction of cutaneous scar tissue and distortion of the cartilage. It can be speculated that the "necrosis" resulted from direct tick effects and body reaction to tick feeding relative to accumulation of neutrophils (Tatchell & Moorhouse, 1970). The dead neutrophils release enzymes from the lysosomes which can cause necrosis of tissues. Besides, Moorhouse (1967) demonstrated lysis of the aural cartilage in guinea pigs infested with Ixodes sp. Grooming provoked by pruritis caused skin abrasions of varied degree. The abrasions bled and/or exuded lymph that encrusted. Four out of five calves developed otitis externa that turned foul when the exudate and dead ticks decomposed and this reaction occurred on one or two occasions in the different animals. The unfavourable environment created by the severe skin reaction caused death of ticks. A lot of flies were attracted to the affected ears while the sleeves remained open but myiasis did not develop. The sleeves were not restored or sealed until complete engorgement of the female ticks was imminent. Continued infestation induced pityriasis in all calves and hyperkeratosis in one. The skin thus became thickened and flaky.

The indirect responses were associated with bleb formation and fibrinous exudate, urticarial eruptions

and pruritis (attributable to release of histamine and allied substances). Blebs, as described by Riek (1962), manifested as papules, 'pustules' and pseudovesicles (simulating blister lesion). The three types of the lesion were seen concurrently at times and developed between or around bite wounds. They appeared within twelve to twenty four hours of infestation. The papules were discrete about two to four millimeters diameter, raised and erythematous at the edges with somewhat blanched centre, and firm in consistency. (1962)
 (The papules illustrated by Riek, however, appear much bigger and they simulate urticarial eruptions). The pustules were the same in structure but could be slightly bigger and appeared entirely grey as if filled with pus (opalescent). Much larger lesions formed on coalescing. No pus escaped on breaking the pellicle which was firmly adherent to the underlying tissue. They progressed to necrosis if undisturbed and healing under the formed scab left durable deep scars resembling aftermath of 'pox virus' infection. The pseudovesicles simulated a blister in appearance but the pellicle was partially free from the underlying tissue. It was anchored to the base at several points instead of being completely separated by the fluid enclosed. Thus the pellicle dome did not all collapse at once on rupture at one spot. The size varied and on coalescing could be one inch or more in diameter. The profuse plasma fluid from ruptured vesicles oozed slowly forming fibrinous clots which became granular encrustations on drying. Removal

of the sleeve with adherent pellicle(s) or deep scraping removed a thick layer of degenerate tissue and exposed raw surface crater(s). This reaction occurred only in previously sensitized calves. The prevalence and severity were most marked in two and mild in another two of the group of five calves. Eczema and baldness were accompanied by a fibrinous exudate from the blebs. However, alopecia also developed in the absence of severe bleb reaction. When undisturbed the lesions dried gradually with the wrinkled pellicle breaking up and flaking. Resolution which was rapid left scars that remained prominent for many weeks. Viewed against a dark pigmented background they were almost colourless initially but gradually became pigmented with time. The nature of the scars indicated that the inflammation involved the deep layers of the skin which would affect the economic quality of the hide.

Widely scattered urticarial plaques (elevated cutaneous nodules, generally round, discrete and firm with standing hair) were detected on two calves at three days post infestation during the sixth infestation only, although it is possible that they were missed at other times. They occurred on uninfested sites, the back, side of the trunk and limbs. They persisted for about twenty four hours and were not painful on palpation nor did the animals appear disturbed at these sites.

5.2. CLINICAL-PATHOLOGICAL FINDINGS

5.2.1. Haematological estimations.

The means and standard deviations of the analysed samples are presented in Figs. 5.5 to 5.13. The erythrocytic indices generally exhibited a gentle decline, especially haemoglobin concentrations (HB) and packed cell volumes (PCV) (Figs. 5.5 & 5.7). However, the red blood cell counts (RBC) (Fig. 5.6) presented a sharp drop at mid-infestation and this was matched by a corresponding rise in the levels of the mean corpuscular volumes (MCV) (Fig. 5.8). The rise indicated a regenerative response and markedly contrasted the levels shown during the first four successive infestations. The mean corpuscular haemoglobin concentrations (MCHC) remained quite stable except during the second and third infestations when the standard deviations were big and the seventh infestation when a steep drop appeared (Fig. 5.9). The big standard deviation of the third infestation coincided with the highest mean levels in HB, RBC and PCV but ^{the} MCHC was declining. The steep drop in ^{MCHC} the at the seventh infestation coincided with the highest level of the mean corpuscular volume and lowest value of red blood cell count. However, the haemoglobin concentration level, though declined, was not the lowest obtained. It is noteworthy that the following readings were higher whilst the corresponding HB and MCV continued to drop and RBC remained almost the same. This erratic effect could be ascribed to technical fault.

FIG. 5.5

Means and standard deviations of Packed Cell Volumes of five calves exposed fortnightly to successive low density infestations of adult Rhipicephalus appendiculatus. (n) was four calves after the 7th infestation.

FIG. 5.6

Means and standard deviations of Red Blood Cell counts of five calves exposed fortnightly to successive low density infestations of adult Rhipicephalus appendiculatus.

FIG. 5.5

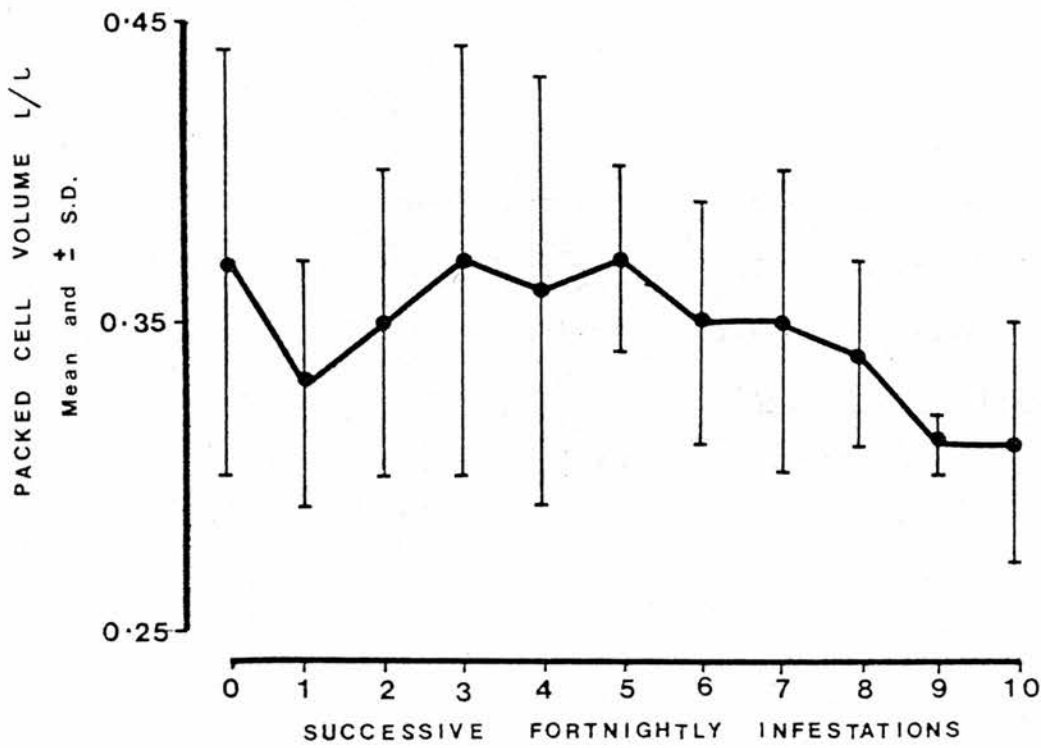


FIG. 5.6

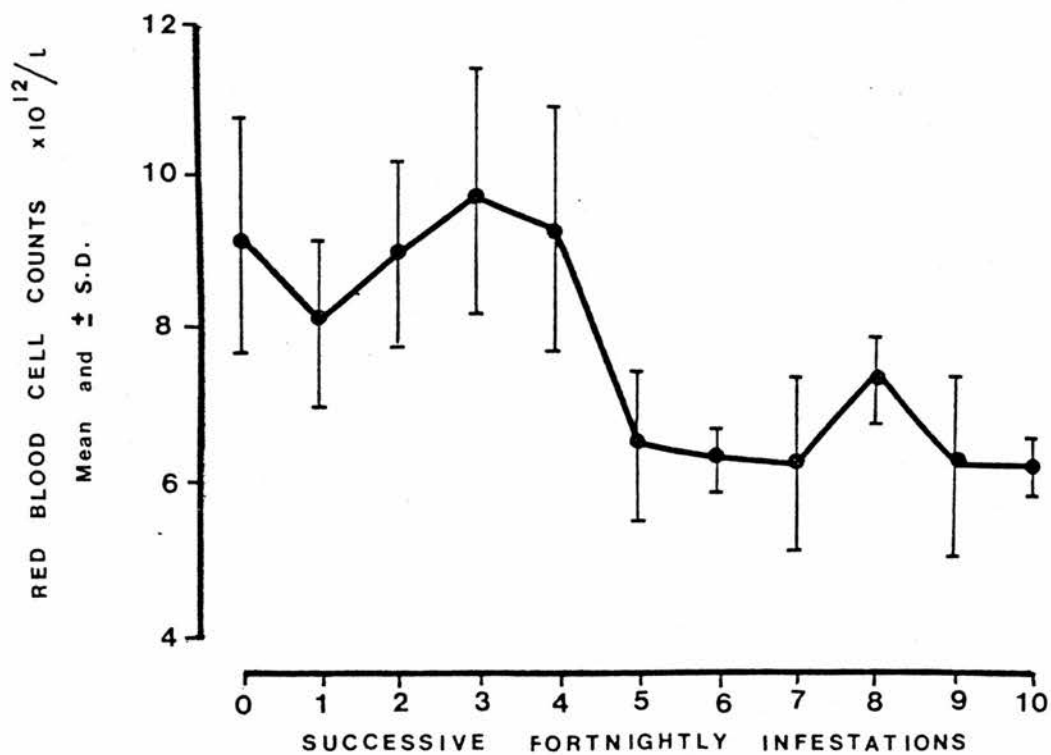


FIG. 5.7

Means and standard deviations of Haemoglobin
Concentrations of five calves exposed fortnightly to
successive low density infestations of adult
Rhipicephalus appendiculatus.

FIG. 5.8

Means and standard deviations of Mean Corpuscular
Volumes of five calves exposed fortnightly to
successive low density infestations of adult
Rhipicephalus appendiculatus.

FIG. 5.7

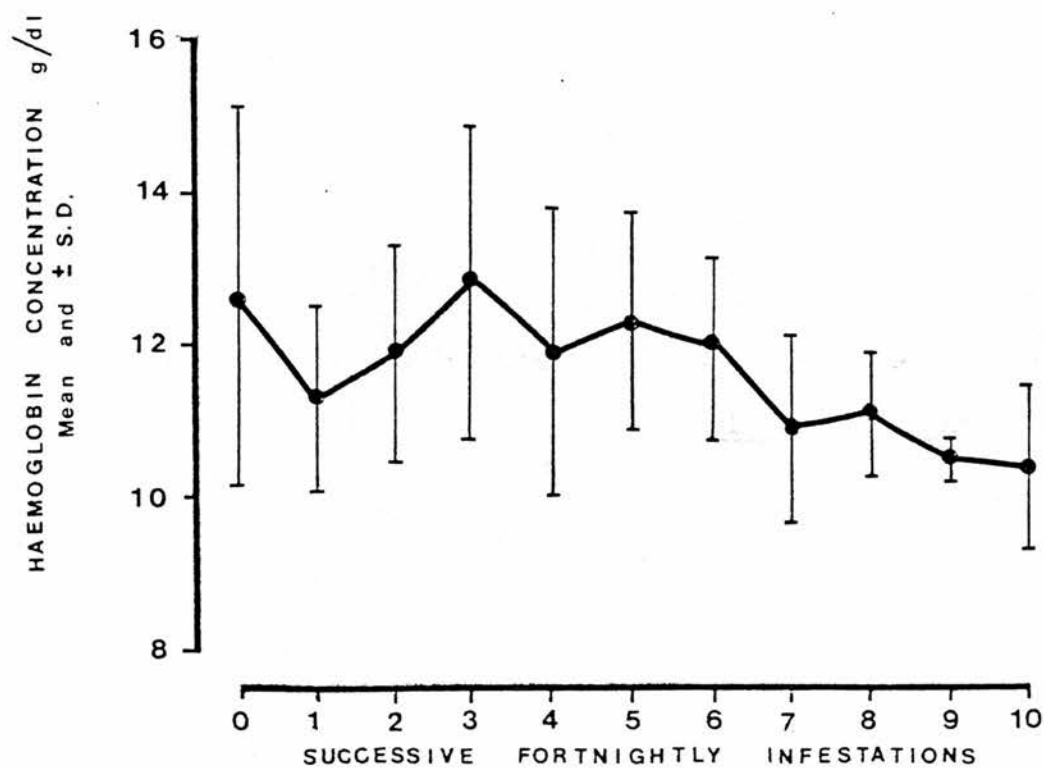
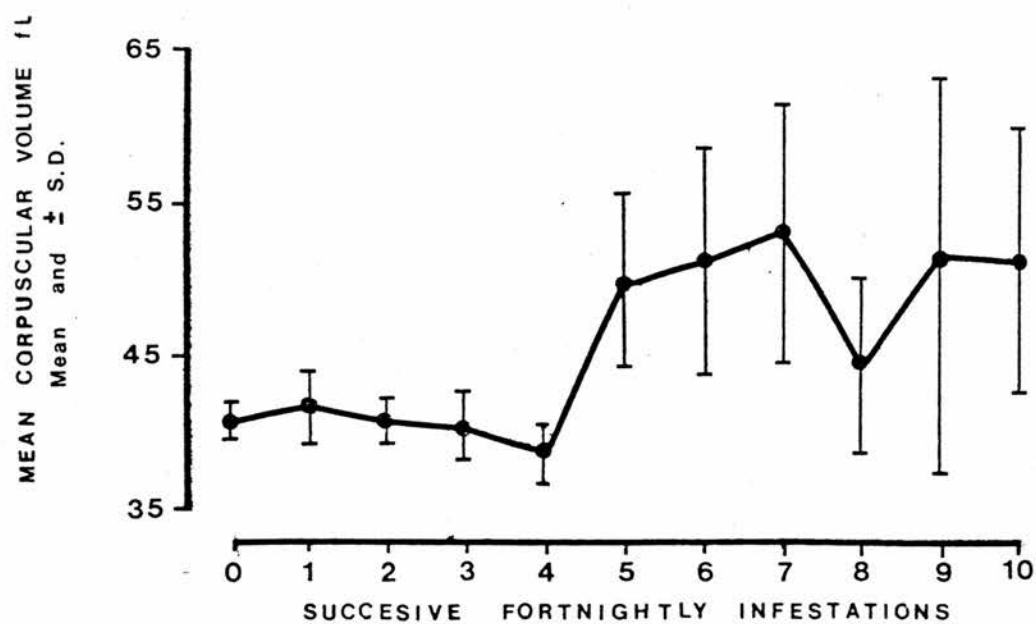


FIG. 5.8



Similarly the drop in the mean corpuscular volume level (Fig. 5.8) during the eighth infestation coincided with an abrupt rise in RBC when the PCV was declining. However, at the seventh, ninth and tenth infestations the standard deviations were relatively big but small in red blood cell count and packed cell volume levels (Fig. 5.5 & 5.6). Nonetheless, the overall variations in the indices were fairly uniform as indicated by the moderately small standard deviations. Thus the low density infestation of adult R. appendiculatus applied, where ticks fed successfully, did not induce critical changes in the erythrocytic parameters. Notwithstanding, the decline indicated an inhibited activity of the haemopoietic system, as the original levels were never recovered during the course of the experiment. However, an increase in the mean corpuscular volume levels (Fig. 5.8) showed that the regenerative potential persisted. The changes observed would suggest a macrocytic normochromic anaemia. Therefore the inhibited responses could be attributed to the effects of repeated infestations of adult R. appendiculatus.

5.2.2. All mean values of the leucocytic series (Figs. 5.10 to 5.12), except the eosinophils (Fig. 5.13), exhibited a similar trend with marked decline and a regression against time that was significant in total white blood cell counts ($r = -0.74$, $p < 0.01$), absolute

FIG. 5.9

Means and standard deviations of Mean Corpuscular Haemoglobin Concentrations of five calves exposed fortnightly to successive low density infestations of adult Rhipicephalus appendiculatus.

FIG. 5.10

Means and standard deviations of White Blood Cell counts of five calves exposed fortnightly to successive low density infestations of adult Rhipicephalus appendiculatus.

FIG. 5.9

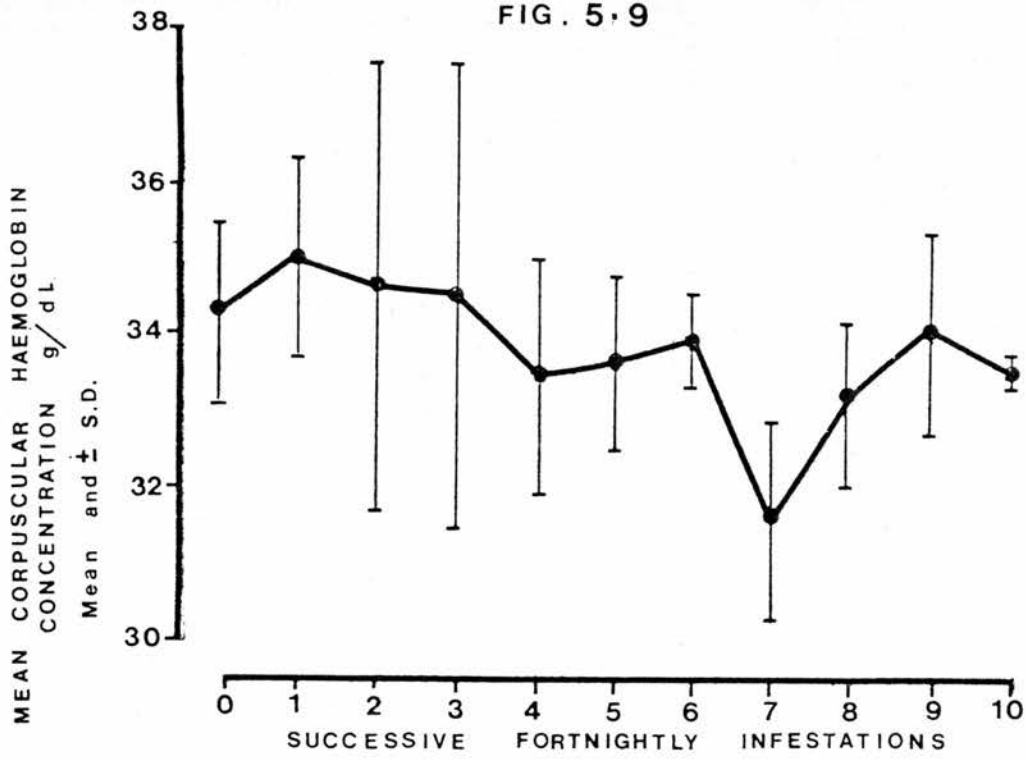
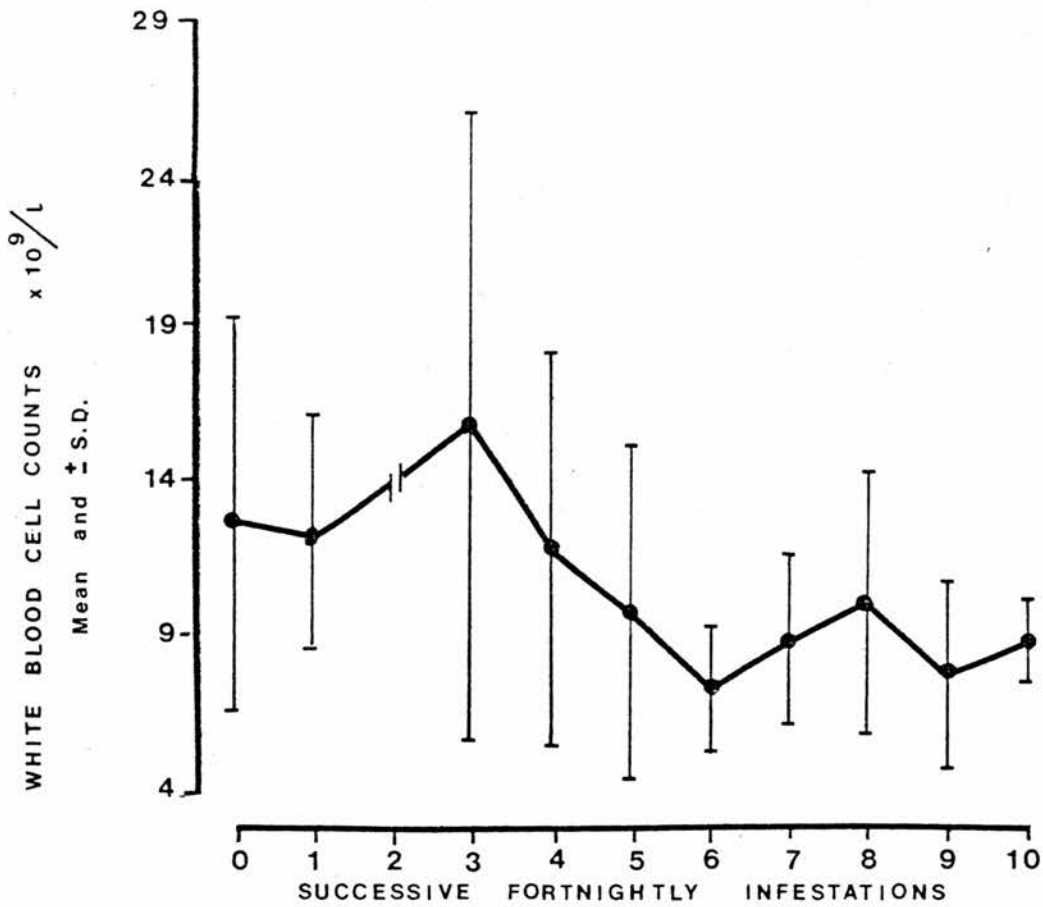


FIG. 5.10



lymphocyte counts ($r = -0.69$, $p < 0.05 > 0.02$) and absolute neutrophil counts ($r = -0.83$; $p < 0.01$, $p > 0.001$). The values for the second infestation were omitted due to technical difficulties which rendered the results unreliable. In most cases the standard deviations were small, though exaggerations appeared at preinfestation, third, fourth and fifth successive estimations. During the infestations the wide variation in responses could be partly attributed to complication of respiratory infection (pneumonia, coughs) and lymph node abscessation.

5.2.3. The mean values of all parameters, except total white blood cell counts (WBC) and absolute lymphocyte counts, were within the normal ranges. The exceptions showed slight elevations which could not be readily explained on inspection of the overall clinical response. However, they could have developed suppurative reactions in internal organs without clinical manifestation but this could not be established because the four surviving calves were not slaughtered for autopsy. Nevertheless, some of these changes coincided with abscessation, attacks of coughs and diarrhoea concurrent with induced pyrexia. An active bacterial complication would be highly possible but the neutrophilia levels were low and occasional. The contribution of lymphocytosis should be considered in chronic suppurative reactions. On several occasions Professor J. Moulton demonstrated multiple internal chronic abscesses in wasted bovines which had shown persistent and marked lymphocytosis during hospitalisation in our clinics at Kabete. In all cases the autopsy and

histological investigations did not reveal any evidence of malignancy associated with lymphosarcoma.

The regression in the overall leucocytic responses indicates that the repeated infestations of adult Rhipicephalus appendiculatus inhibited the circulating leucocyte levels, which agrees with studies made by Williams et al., (1977) and Williams (1978) on the effects of high and protracted infestation of ticks on the blood composition of steers and van Rensburg (1959) on rhipicephaline toxicosis in cattle. The absolute eosinophil count levels were relatively low, thus not indicative of parasitism, and the cells were found in only 47% of the estimations made. As described before (C.4.3.1) the low levels could be associated with the rapid re-establishment of the equilibrium of blood histamine after detachment of ticks (Riek, 1955) and delay in blood sampling when the peak stimulus of histamine release into the circulation has waned (Schalm et al., 1975).

5.2.4. Analysis of variance applied to the haematological data revealed significant differences between the uninfested control group and the infested groups in all parameters (Tables 4.1 to 4.9) except the absolute neutrophil counts (Table 4.7). The group (II) was not included in two analyses because no eosinophils were found (Table 4.9). The frequency and levels of significance have been described in section C.4.3.2.

FIG. 5.11

Means and standard deviations of absolute Neutrophil
Cell counts of five calves exposed fortnightly to
successive low density infestations of adult Rhipicephalus
appendiculatus.

FIG. 5.12

Means and standard deviations of absolute Lymphocyte
Cell counts of five calves exposed fortnightly to
successive low density infestations of adult Rhipicephalus
appendiculatus.

FIG. 5.11

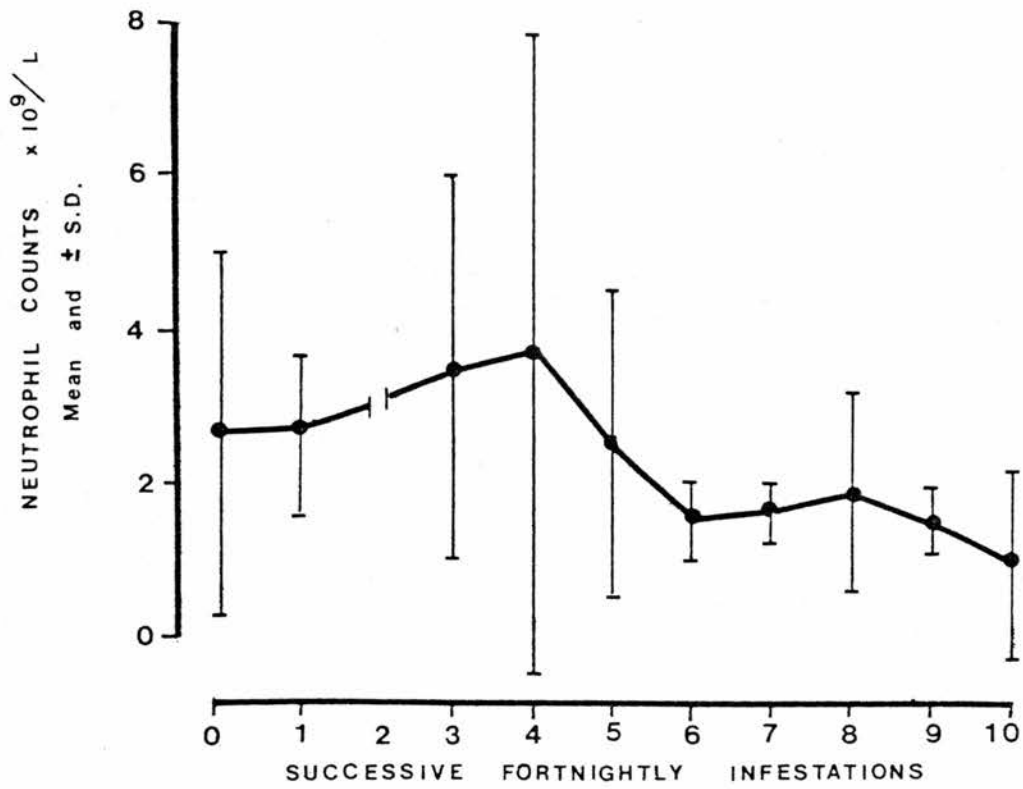
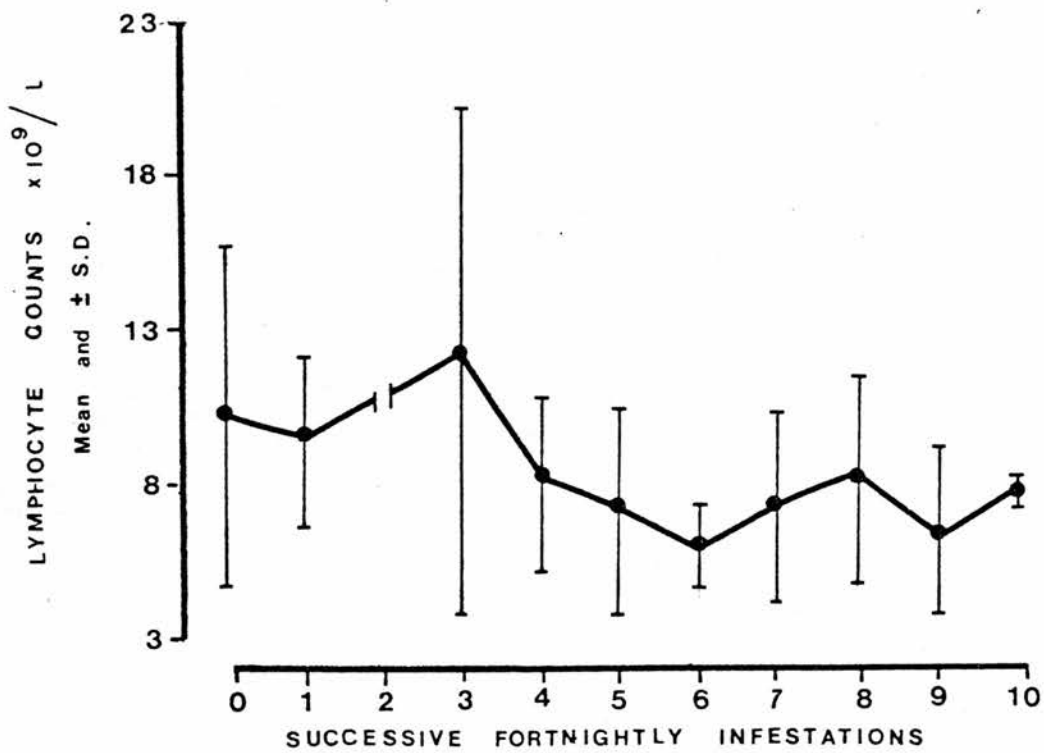


FIG. 5.12



5.2.5. Duncan's New Multiple Range test (DNMRT) applied to data showing significant differences between groups resolved significant subsets in which this group (II) (infested with low density adult R. appendiculatus) responded independently and similarly to other experimental groups and also overlapped between subsets (Tables 4.10 to 4.17). The interpretation of the analyses derived by the DNMRT is described in section C4.3.3. Thus the test associated this group (II) closest to group (I) (uninfested controls) followed by groups (IV) (infested with nymphs) and (V) (infested with larvae) and furthest to group (III) (infested with high density adults). The ranking showed the group (II) assumed the highest magnitude in individual parameters at 50% of the analyses in each of packed cell volumes (PCV), and haemoglobin concentrations (HB) (Tables 4.10 & 4.12); at greater than 50% of the analyses in each of mean corpuscular volumes (MCV), absolute counts of lymphocytes and eosinophils (Tables 4.13, 4.16 & 4.17); and at less than 50% of the analyses in each of red blood cell counts (RBC) and total white blood cell counts (WBC) (Tables 4.11 & 4.15). Conversely the lowest ranking was revealed once for each of the MCV and mean corpuscular haemoglobin concentrations (MCHC) parameters (Tables 4.13 & 4.14). These derivations match the values illustrated in the corresponding figures (5.5 to 5.13).

5.2.6. Biochemical estimations

The means and standard deviations of samples taken

FIG. 5.13

Means and standard deviations of absolute Eosinophil
Cell counts of five calves exposed fortnightly to
successive low density infestations of adult Rhipicephalus
appendiculatus.

FIG. 5.14

Means and standard deviations of Serum Protein
levels of five calves exposed fortnightly to successive
low density infestations of adult Rhipicephalus
appendiculatus.

FIG. 5 · 13

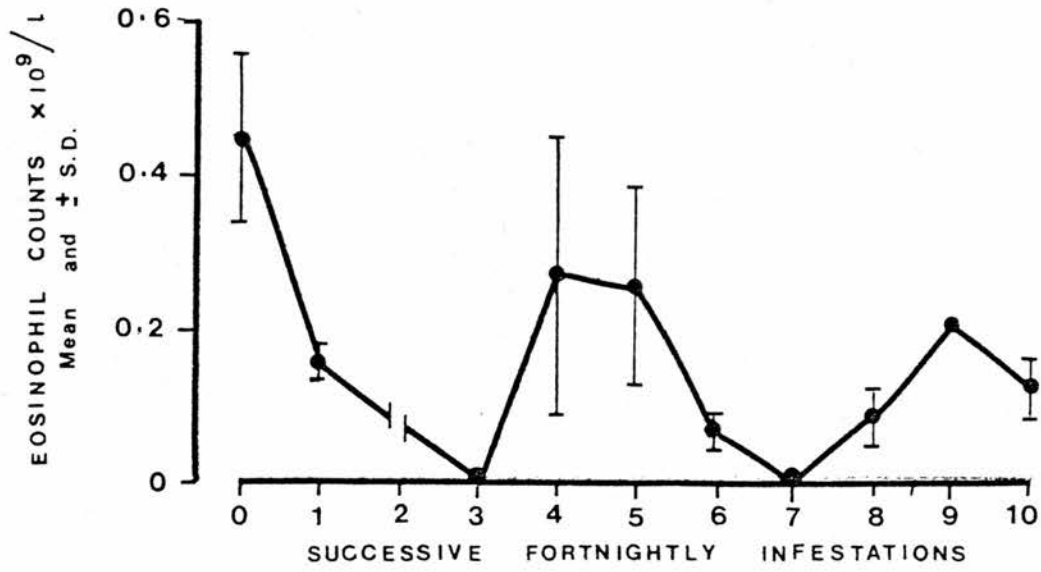
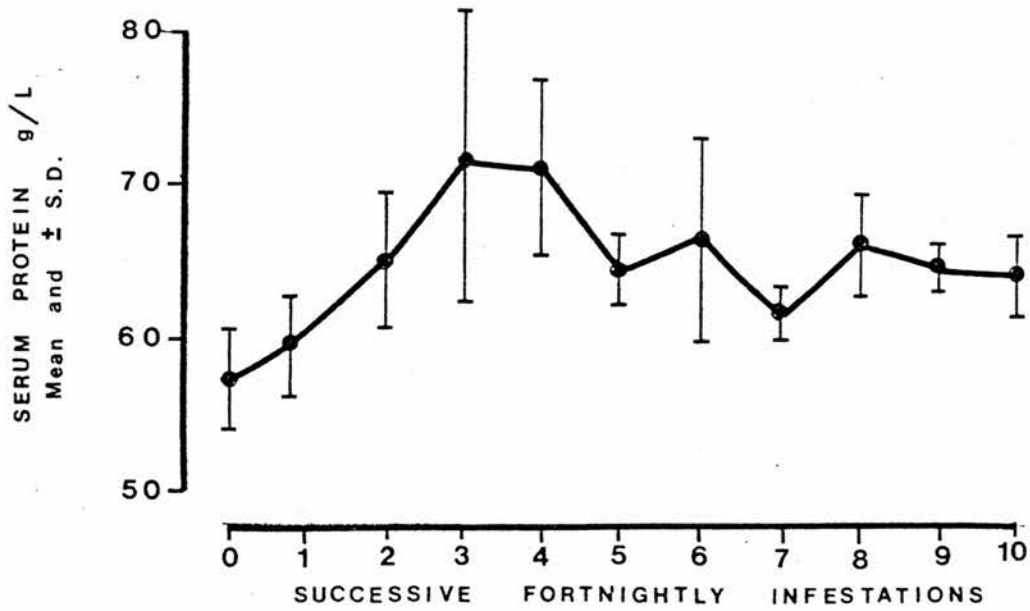


FIG. 5 · 14



during successive infestations are presented in Fig. 5.14 to 5.16. The missing values for pre-infestation estimation of serum glucose (S.G.) and for ninth and tenth infestations of immunoglobulins (IG) were not obtained for technical reasons.

The mean values of serum protein levels (S.P.) were within the normal range (Coles, 1974), for most estimations, though initially marginal and subnormal levels were obtained. The reason for this anomaly was not clear since tick feeding could not have been responsible and the levels rose subsequently when the tick feeding was established. The standard deviations were generally small. The highest mean values coincided with similar values in the immunoglobulin levels, but these were followed by a gentle and progressive decline. The mean values of the immunoglobulin levels were well above the minimum optimal level (Irwin, 1974) and the standard deviations were small. However, the successive values showed^a/similar trend to serum protein levels (S.P.), but IG. exhibited more uniformity in the levels (Fig. 5.15). It could be suggested that the rise in the immunoglobulin levels obtained after the second fortnightly infestation was attributable to antigenic stimulus, though this is not convincing since no significant differences were revealed between infested and uninfested control groups except for the pre-infestation estimations. The differences revealed at the pre-infestation estimations could be a simple reflection of levels of maternal immunoglobulin in

FIG. 5.15

Means and standard deviations of Immunoglobulin levels of five calves exposed fortnightly to successive low density infestations of adult Rhipicephalus appendiculatus. (Estimations for ninth and tenth exposures missing for technical reasons).
(n) was four calves after 7th infestation.

FIG. 5.16

Means and standard deviations of Serum Glucose levels of five calves exposed fortnightly to successive low density infestations of Rhipicephalus appendiculatus .
(Pre-exposure figures missing for technical reasons).

FIG. 5.15

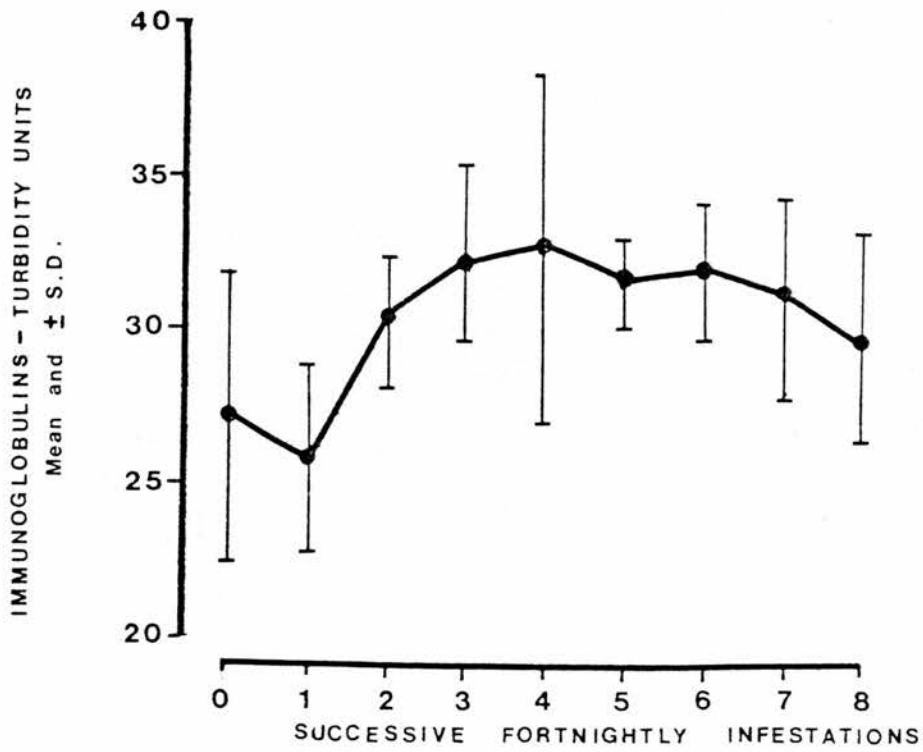
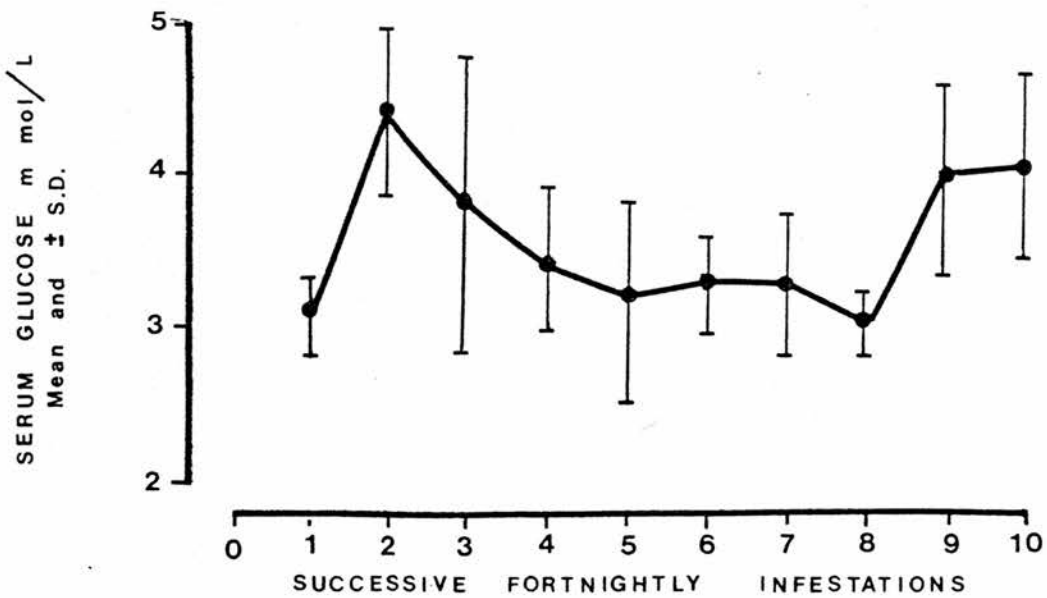


FIG. 5.16



the younger calves. In contrast serum glucose (S.G.) showed consistently high normal mean levels that varied within narrow limits illustrated by the small standard deviations (Fig. 5.16). The depression in the mean levels of serum glucose from the third to eighth successive infestation was associated with marked loss in body condition, persistent pyrexia and highest mean infestations of adult R. appendiculatus applied (Fig. 3.1). Thus the reduction in the tick density subsequently was accompanied by a rise in the glucose levels. The tick values for the last two infestations are missing (Fig. 3.1) as they were incomplete on account of the behavioural reactions which caused losses of ticks at different stages of repletion.

5.2.7. Analysis of variance similarly revealed significant differences between the uninfested control group (I) and the infested groups, including this group (II), in all three parameters estimated (Tables 4.18 to 4.20). However, the differences were detected only once in the pre-infestation values of immunoglobulins and in less than 40% of the estimations of serum protein and serum glucose.

5.2.8. Duncan's New Multiple Range test (DNMRT) as described earlier (C.4.3.3) eliminated the significant differences between groups by resolving significant subsets in which the group (II) responded independently and similarly to other experimental groups and also overlapped between subsets (Tables 4.21 to 4.23). Thus

the test associated the group closest to groups (IV) (infested with nymphs) and (V) (infested with larvae) followed by (III) (infested with high density adults) and (I) (uninfested controls) in descending order. On three occasions during infestations (Tables 4.21 & 4.23) the group (II) carried the highest rankings. The relegation of group (I) to lower rankings in serum glucose and serum protein in contrast to this group (II) indicated greater responses. However, lack of significant differences in immunoglobulin levels during tick feeding weakens the suggestion made.

5.3. AUTOPSY

One calf (T.5) died after protracted illness of chronic bronchopneumonia confounded by inanition effects. The morbid changes found were as follows. The carcass was extremely emaciated. Epidermal lesions induced by infestations and the presence of attached ticks were prominent. The thoracic cavity showed pleuritic adhesions between lungs and sternum. The lungs showed marked pneumonic changes in the left diaphragmatic lobe and bilaterally in the anterior lobes. The trachea, bronchi and some bronchioles were filled with white froth (contributory to terminal dyspnoea). Cut surface revealed the complete consolidation of/entire left lung with pus in the

bronchioles. The right diaphragmatic lobe showed extensive emphysema. The heart was slightly pale. The lymph nodes showed enlargement in the mediastinal and parotids and the left parotid was abscessed. Abdomen showed nothing significant in the peritoneal cavity. Spleen was much reduced in size. Liver was slightly enlarged. Kidneys carried numerous cortical petichiae (tiny haemorrhages). Bacteriological investigations revealed a pure culture of Corynebacterium pyogenes from the lung and abscessed lymph node samples. It was concluded that the main pathological syndromes were pneumonia and terminal septicaemia. Thus the final diagnosis of cause of death was a subacute suppurative bronchopneumonia of C. pyogenes origin. In retrospect the development of the pneumonia is attributable to primary abscessation in the parotid lymph node from where the C. pyogenes spread to the lungs (C.5.1.5). The effects of the tick infestation were therefore obscured by the severe pneumonia and the terminal septicaemia. The only specific tick effects were found in the skin lesions but were not severe enough to be associated with cause of death.

5.4. SUMMARY OF SIGNIFICANT FINDINGS

Calves reacted to the successive low density infestations of Rhipicephalus appendiculatus by evincing definite physical and clinical-pathological changes

manifested as follows. 1) Immediate reactions to commencement of tick feeding were associated with pain and irritation but were generally transient in first infestations and more sustained during repeated infestations. 2) Rapid deterioration in body condition, demeanour and physique was confounded by concurrent secondary complications, e.g. secondary pneumonia 3) Pyrexia of varied degree and protracted course associated with engorgement of female ticks and hypersensitization was induced. Thus mono and diphasic responses emerged in primary (first) and secondary (repeated) infestations respectively. The mean pyrexia levels progressively regressed to normal temperature during successive infestation ($r = -0.86$, a.m. & -0.89 , p.m.; $p < 0.001$). 4) Other circulatory disturbances elicited considerable erythema, oedema, conjunctival congestion and excessive secretions from the visible mucous membranes. 5) Lymphadenopathy in regional drainage nodes was a constant feature and was complicated by abscessation. The subdued reaction of the lymph nodes occurred after several successive infestations of R. appendiculatus. 6) Chronic suppurative bronchopneumonia associated with Corynebacterium pyogenes organisms was diagnosed in one calf. The pneumonia was possibly exacerbated by spread of the organisms from an abscessed lymph node.⁷⁾ Digestive system activity was maintained despite marked deterioration in clinical condition. 8) Pruritis, specially intense on repeated exposure and its complications, was constant and associated predominantly with

feeding of female ticks 9) Severe reactions of aural tissue developed and led to transformation and distortion, e.g. exudation, necrosis, alopecia, keratosis, curled/wrinkled pinna. 10) The hypersensitivity state manifested by bleb formation and urticarial eruptions and increased exudation developed. The clinical manifestations appeared during secondary exposures and inhibited tick performance. 11) Levels of infestation did not induce critical derangement in haematological and biochemical parameters but caused appreciable decline from the initial values which were not recovered. Thus significant regression against time was revealed in total white blood cell counts and absolute counts of lymphocytes and neutrophils. Bone marrow activity was maintained despite the protracted exposure. 12) Statistically significant differences were revealed between the uninfested controls and the infested groups, including this group (II), in clinical-pathological estimations. 13) Autopsy on the dead calf revealed a chronic suppurative bronchopneumonia associated with Corynebacterium pyogenes organisms. The pneumonia was linked with an abscess in a parotid lymph node caused by the same organisms.

CHAPTER 6

EXPERIMENT (III): RESPONSES OF FIVE CALVES EXPOSED WEEKLY
TO SUCCESSIVE HIGH DENSITY INFESTATIONS OF ADULT
RHIPICEPHALUS APPENDICULATUS

Five calves aged two to four months and previously unexposed to ticks were clinically healthy at the start of the first infestation. The mean numbers of ticks applied and the engorged females recovered in the successive infestations are presented in Figure 3.2. The five calves reacted severely to the high density infestations of adult R. appendiculatus applied which caused 60% natural deaths. The calves were systematically examined daily during the infestations. Restraint against grooming was applied during infestation. Blood samples for clinical-pathological estimations were collected at each successive infestation. Autopsies were performed on three dead and two slaughtered calves and the gross pathological changes recorded. The responses induced are presented under three major headings (C.6.1, 2, 3).

6.1. PHYSICAL (CLINICAL) SIGNS

The signs studied followed the same pattern as for experiment (II) (C.5.1.1 to 5.1.8). The first infestations did not cause^a/significant immediate response to attachment of the ticks. However, the successive infestations induced various signs at attachment which

were associated with pain and irritation, as described for experiment (II). Other signs developed as the ticks continued feeding. Some of the reactions were transient, e.g. interruption of feeding, frequent urination whereas others persisted throughout the infestation and longer, e.g. pruritis, pyrexia, lymphadenopathy. The signs varied in onset, severity, prevalence and persistence in the individual calves during the successive infestations. The observed clinical manifestations are described under various body systems as follows.

6.1.1. The body condition was assessed as before based on the appearance of the coat, prominence of bones, hollowness of paralumbar fossae and eyes, and the muscle cover over shoulders and hindquarters. Decline in the condition started during first and second infestations in different calves. Four out of the five calves evinced progressive decline throughout the experiment culminating in irreversible cachexia before death and this was confounded by the appearance of anaemic changes. However, one calf (T.22) that survived the planned ten exposures recovered steadily through the last three infestations. This was attributed to vigorous grooming facilitated by slack restraint (because of extreme weakness), marked reduction in the recovered numbers of successfully engorged female ticks (Fig. 3.2) and ability of the calf to feed keenly. The density applied during the tenth infestation was limited by tick shortage. This recovery rate was analogous to the observation of rapid

and complete recovery following deticking reported by Philip et al., (1935).

Four calves showed progressive deterioration in demeanour and physique leading to extreme depression, feeble response to immediate surroundings, extreme weakness and prostration terminally. The extreme weakness affected the normal function of both the musculoskeletal and digestive systems. The signs commonly observed were unsteady gait, frequent recumbency accompanied by difficulty or reluctance to rise, abnormal head/neck posture, partial closure of the eyelids and drowsiness and feeble movements in prehension and mastication. Thus the demeanour and physique were greatly influenced by critical anaemia and inanition. Calves remained prostrate for up to three days before death.

6.1.2. The cardiovascular system, as for experiment (II) (low density infestations of adults), was seriously affected and the features recorded followed the same pattern. Pyrexia was induced in all calves to varied degrees in the p.m. rectal temperature during most infestations. However, the morning temperatures scarcely rose above the critical level (39.3°C) after the fifth successive exposure. These relatively low temperature levels, when compared with the response in the uninfested controls indicated an inhibition of the heat regulation mechanism. During the first four to six infestations individual calves attained severe pyrexia levels ($39.3^{\circ} + 2.1^{\circ}\text{C}$ or

FIG. 6.1.

Mean weekly Rectal Temperatures of five calves exposed weekly to successive high density infestations of adult Rhipicephalus appendiculatus compared with uninfested controls. Arrow marks normal critical temperature; (n) value dropped to two in last two infestations. (n) = number of calves in the group treated.

FIG. 6.2.

Mean daily Rectal Temperatures of five calves exposed to first high density infestation of adult Rhipicephalus appendiculatus compared with uninfested controls. Arrow marks critical normal temperature; (n) value dropped to two in last two infestations.

FIG. 6.1

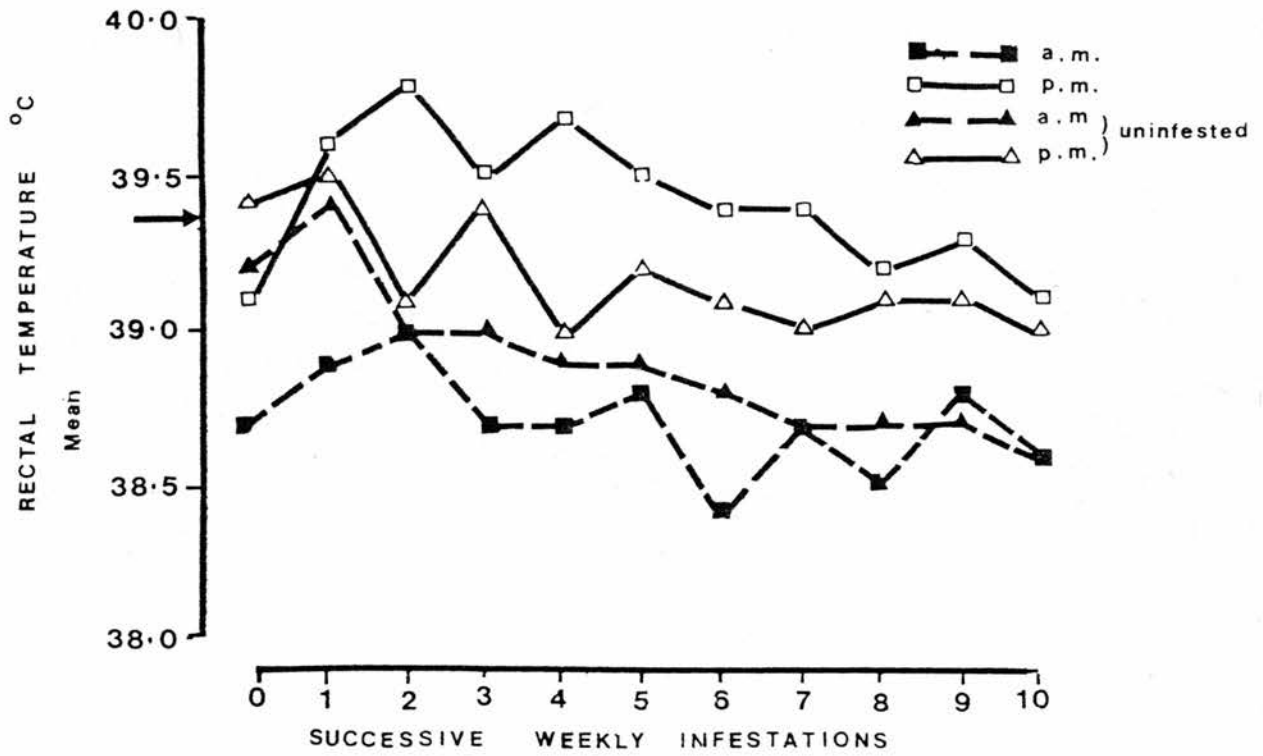
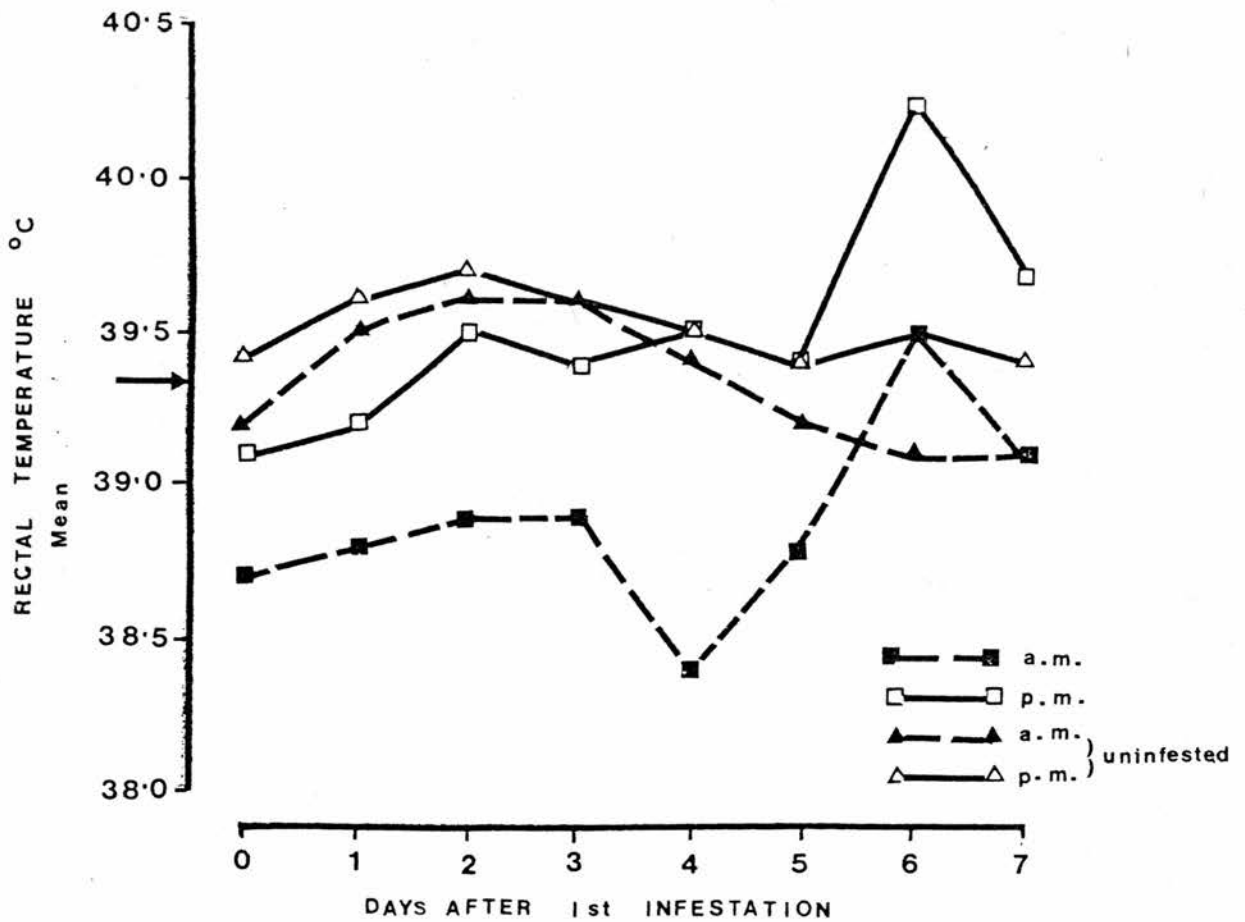


FIG. 6.2



more). Normal or only slightly elevated temperatures were found during the infestations immediately preceding death and coincided with critical body condition. During the two days preceding death the prostrate calves ran subnormal temperatures. However, transient subnormal rectal temperature (36°C) and poor vasomotor tone (determined by palpation of body extremities) appeared in one calf that developed haemoglobinuria (dark-red discoloration but contained no red blood cells on centrifugation). The composition of the urine is described later (C.6.1.5). The mean weekly rectal temperature responses reflecting some of the above features are presented in Fig. 6.1. The values exhibit slight mean pyrexia levels in p.m. temperatures which declined steadily to practically normal levels whilst the means of the a.m. temperature remained quite normal. The trend in the decline, whilst similar to reactions in experiment (II), did differ from responses exhibited in experiment (VI) (Fig. 10.1, concurrent three instar infestations). The mean rectal temperatures in Fig. 10.1 suddenly reverted to normal despite the high infestation challenge. This observation further supports the suggestion of inhibition of the heat regulating mechanism. However, the steady decline in the mean levels manifested in this experiment (III) could also be attributed to reduced mean number of ticks applied (Fig. 3.2) and relatively lower recovery of engorged females and exhaustion of the calves before death. As stated above the pyrexia during the infestations preceding death was only slight and deaths

commenced round the sixth through to tenth infestation, hence depression in the mean temperatures then. It is interesting to note the development of a similar trend in experiment (II) (Fig. 5.1) where death associated with direct tick effects did not occur. The idea of inhibition of the heat regulating mechanism (or tolerance) was reflected in the appearance of pyrexia levels in only p.m. temperatures of four exposures. Furthermore, the a.m. temperature levels were well below those shown by the uninfested controls. The exhaustion of the calves in this experiment (III) can be ascribed to extreme loss of body condition leading to depressed muscular, digestive and cardiovascular activities connected with generation and maintenance of body heat. These changes had a contributory role in the development of prostration relative to the most direct initial effects of blood loss and pyrexia. The mean rectal temperatures of the uninfested control group (I) generally were within the normal range and the anomalies in the initial weeks were attributable to non specific syndromes. The results in Figures 6.2, 6.3 & 6.4 demonstrate the pattern of responses to primary, secondary exposures and the remission in the mean pyrexia levels during the sixth infestation. The response to primary infestation (Fig. 6.2) showed a gradual slight pyrexia in the mean daily temperatures culminating in sharp peaks (a.m. & p.m.) that coincided with engorgement of the female ticks. This distinctly contrasted the response at the corresponding period in the uninfested control group compared in the same figure. The response

FIG. 6.3

Mean daily Rectal Temperatures of five calves exposed to second successive weekly high density infestation of adult Rhipicephalus appendiculatus compared with uninfested controls. Arrow marks normal critical temperature; (n) value dropped to two in last two infestations .

FIG. 6.4

Mean daily Rectal Temperatures of five calves exposed to sixth successive weekly high density infestation of adult Rhipicephalus appendiculatus compared with uninfested controls. Arrow marks normal critical temperature; (n) value dropped to two in last two infestations .

FIG. 6-3

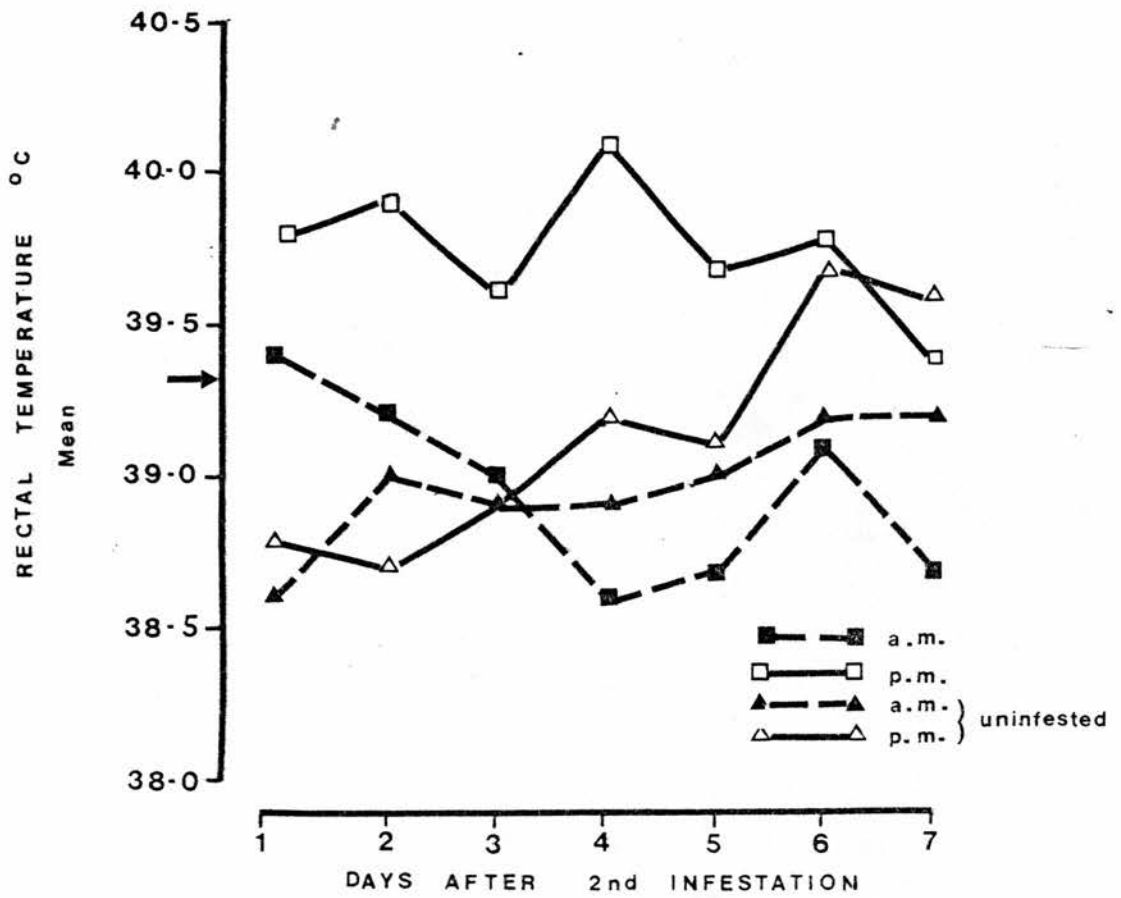
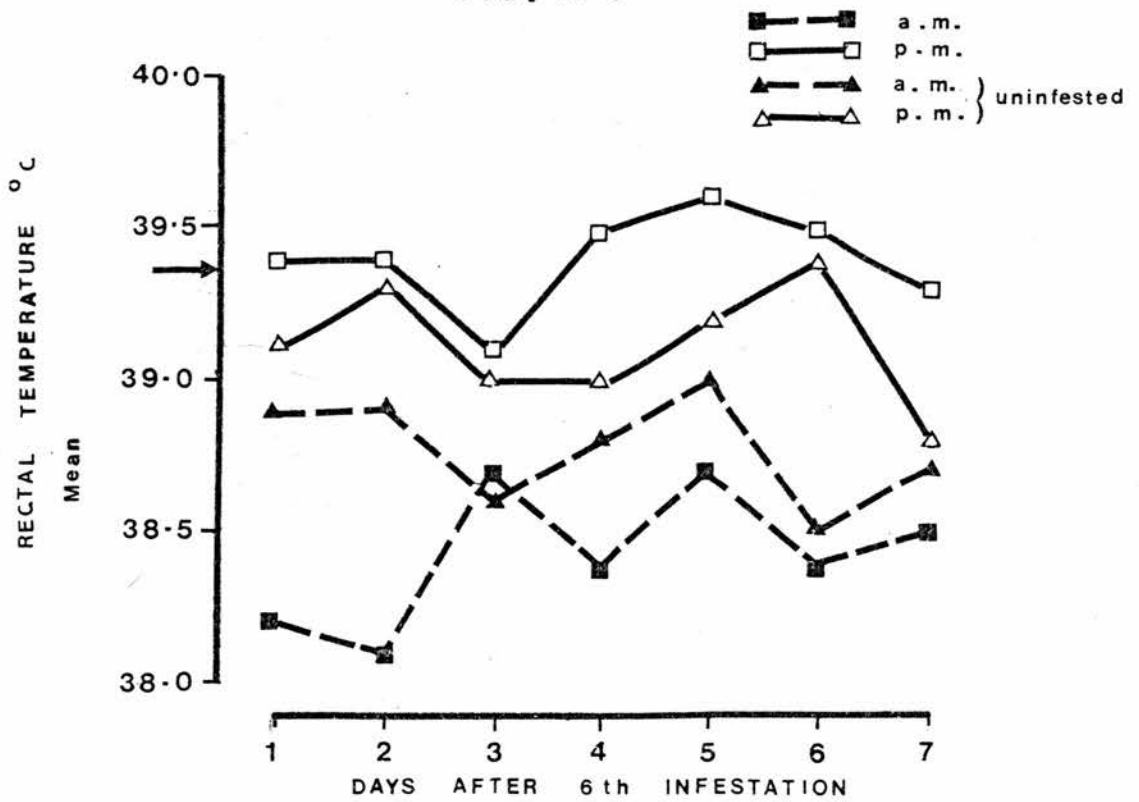


FIG. 6-4



also differed from the response to the corresponding infestation in experiment (II) (Fig. 5.2, infestations of low density adults). Only one sharp peak appeared in Fig. 6.2 despite a much higher density of ticks applied whereas experiment (II) produced two rises, the first was unrelated to engorgement activity. Response in previously sensitized hosts (Fig. 6.3) showed an early pyrexia (p.m.), in the mean daily rectal temperatures during the second successive exposure, that persisted throughout infestation and elicited different peaks with the last coinciding with engorgement of females. In contrast, the corresponding mean daily temperatures (p.m.) of the uninfested control group (I) compared in Fig. 6.3 showed delayed pyrexia levels associated with non-specific factors. The response to infestation when the mean weekly pyrexia levels showed a remission is illustrated in Figure 6.4. The diphasic rise was elicited and the peak of the second coincided with engorgement time of the female ticks, but the degree of the pyrexia was slight throughout. It is noteworthy that the mean a.m. temperatures were much lower than in previous infestations. However, the uninfested controls showed two insignificant pyrexia peaks and the second appeared at/^adifferent time as illustrated in Fig. 6.4. The temperature levels illustrated for the group further support the suggestion of inhibition of the body heat regulating mechanism as compared with the uninfested control group.

Heart and pulse function was generally only slightly affected. However, one calf (T.22) showed elevated rates (96 to 132/minute) which persisted through seven successive infestations (i.e. 4th to 10th), in this case associated with anaemic changes. Conversely the transient decline associated with haemoglobinuria occurred when the pulse was barely perceptible and the vasomotor tone poor. As expected decline in rates (42 to 48/minute) and force of heart beat and pulse developed during the two days before death. However, poor vasomotor tone was found at other times of extreme clinical weakness. Another sign associated with anaemic changes was the jugular pulsation detected in two out of five calves for about five and six infestations. The pulsation extended to the upper third of the neck when the head was held level with the withers in normal standing posture. Normally the pulsation of the jugular vessels is observed in the lower half of the neck. However, the jugular vessels were not distended as is found in chronic congestive cardiac syndromes. Mucous membranes and secretions were for most of the time normal in their characteristics (pink, moist and glistening). Nevertheless, all showed congested conjunctivae, especially on the infested side(s) during most infestations and/or pallor at different stages. Pallor appeared first in the second and fourth exposures in one and four calves respectively and persisted until death or disappeared on recovery. This sign indicates depressed levels of circulating red blood cells. The

calf (T.22) that survived regained colour from the eighth exposure and this was accompanied by improvement in the anaemia picture. Extremely weak animals did not drink sufficiently without assistance and therefore showed signs of dehydration. The associated signs as manifested/sunken eyes, reduced moisture and lustre of the mucous membranes, passage of granulated faeces with low moisture, reduced urination and skin elasticity. However, extreme emaciation and general muscle weakness can be accompanied by some of these signs in the absence of dehydration, but further laboratory tests on blood and urine could assist in the diagnosis of genuine dehydration. Abnormal secretions and discharges appeared from eyes, nostrils and mouth and varied in consistency from serous/mucoid to mucopurulent and orally frothy saliva. As observed in experiment (II) (C.5.1.3.). two calves secreted copious saliva during three and four infestations without apparent cause since careful examination of the head and mouth revealed no lesions that could be implicated. Thus more than one type of secretion/discharge were simultaneously produced in individual calves. Erythema and oedema, mostly checked in the skin, were observed at different times and for varied periods in individual calves during infestations. Erythema was constant around attachment sites and contiguous areas. However, marked generalised erythema which intensified with grooming appeared in one calf (T.23). This calf bled profusely from horn stumps left after the horns

were broken through by frantic grooming. Bleeding took long to stop. Grooming is associated with elevated blood histamine levels and since the bleeding coincided with grooming it can be suggested that the delayed blood coagulation was caused by high histamine levels in the circulation. This would conform to the observation that blood coagulation was impaired during challenge of sensitized guinea pigs with egg albumin (Code, 1939). Code (1939) found elevated blood histamine levels on challenge. Slight to moderate oedema was elicited in the ears, but was subdued in latter infestations in four out of the calves. The affected ears became thickened, droopy and produced increased exudation from bite wounds. The dried exudate and necrotic epidermal tissue formed thick encrustations. Occasionally oedema appeared round eyelids and periorbital, intermandibular and sub-parotid regions. It is noteworthy that the calf (T.23) which developed generalised erythema did also evince multicentric oedema in two secondary exposures. Both signs were noticeable by the first to second day post infestation and persisted to the end of female tick feeding. Nevertheless, facial and intermandibular oedema subsided much earlier but the generalised erythema persisted a little longer. Cyanosis (blue discoloration) of the ears developed in two calves in the course of severe reactions that caused death and degeneration of ticks in situ. The cyanosis indicated poor circulation in the tissues and low oxygen tension associated in this case with necrotic changes and

impaired circulation from the oedema. Hypersensitivity reactions, associated with severe circulatory disruption mediated through histamine release in local tissues caused marked exudation from the infested ears.

6.1.3. Lymphatic system changes occurred in local drainage nodes and were more marked in the parotids than the submaxillary and were seldom seen in other superficial lymph nodes. The maximum enlargement attained was three to five times the pre-infestation size. Enlargement was detectable on the second day post infestation (dpi) and persisted throughout the infestation. The response was readily induced in successive exposures but the severity declined during the infestations preceding death when the clinical condition became critical and/or when the tick burden was markedly reduced. This inhibited response was also observed in experiment (II) (C.5.1.4) and further supports the observation made by van Rensburg (1959) on steers infested predominantly with Rhipicephalus appendiculatus. It is most likely that the low grade reaction was related to degeneration of the lymphoid follicles as reported by van Rensburg (1959). Regression to the original size occurred within eight to twelve days after complete engorgement of the female ticks. However, one calf sustained a persistently enlarged parotid lymph node for three consecutive weeks. Subsequent exploratory centesis revealed abscess formation (pus) and the aspirated pus produced a pure culture of Corynebacterium bovis bacteria. The lesion was associated with concurrent leucocytosis

and neutrophilia indicative of^a/response to bacterial infection. The calf was then withdrawn from the experiment after the sixth infestation on confirmation of the abscessation. The withdrawal was planned for cases which developed complications not easily controlled and bound to bias the responses to direct tick effects. The same complication was confirmed in one calf (T.5) as early as the second successive low density infestation of R. appendiculatus (C.5.1.4). However, the present case did not exhibit frank pneumonic symptoms though autopsy revealed significant pneumonic changes.

6.1.4. Respiratory system showed serous to thick mucopurulent nasal discharge sometimes concurrent with coughing in three calves. The discharge was the most consistent change during the first six and the ninth infestations. The elevated respiratory rates (36 to 66/minute) accompanied by forceful expiration and expiratory grunt occurred in two calves out of five. The tachypnoea coincided with severe loss in condition, exercise intolerance and/or anaemia. However, at terminal recumbency stages breathing became deep and slow associated with bradycardia (42 to 48 beats/minute).

6.1.5. Digestive system function in all respects was normal in^{the}/first two exposures and animals were keen on all feeds given. Thereafter the appetite remained generally keen but somewhat selective for concentrates and milk as the clinical condition markedly declined.

There were short intermittent periods of inappetence associated with pain, irritability or severe weakness. Drastic reduction in feed intake, especially hay, was observed and attributed to poor body condition, extreme weakness that led to prolonged recumbency and feeble eating (prehension and mastication). Desire to feed persisted to almost the last day or so as shown by hand feeding of prostrate animals which managed to chew the stuff given. Nevertheless, gastrointestinal activity persisted in most cases, i.e. rumination, ruminal motility and defaecation. Rumen atony, however, predisposed one calf to bloat through feeding keenly on milk and concentrates. Copious drinking was evident in two out of five calves at various times in the morning. It appeared they were too weak to reach the troughs during the night without assistance after being recumbent for several hours. Changes in appearance of dropped faeces often showed constipation (scanty, dry and granulated), voluminous and mucus containing evacuations. These appeared during marked pyrexia and probable dehydration phases.

Urinary system showed frequent urination often during engorgement of female ticks in different infestations. The reaction was also an immediate response to attachment especially in the secondary exposures. Urine was visibly normal all the time but occasionally became abnormal. Haemoglobinuria for two days was

detected once in one calf (T.23, elicited generalised erythema) about the 3rd/4th day of third successive exposure to adult R. appendiculatus. One thousand adult ticks (male & female) had been applied to the calf compared with the mean number of 1500 ticks for the group (Fig. 3.2). Urinalysis revealed specific gravity (sp.gr.) 1.041 (normal), pH 5.4, WBC (lymphocytes and neutrophils), epithelial cells, casts and crystals (triple phosphate), colour, deep yellow (orange). The sample caught on the first day of detection was not analysed in detail but centrifugation left a dark reddish brown supernatant and the smear from the sediment showed no erythrocytes nor bacteria. Thus the discoloration was not due to haematuria. Repeated haematological samples did not reveal Babesia sp. or other associated haematogenous parasites capable of inducing intravascular haemolysis. Close inspection of the results for the calf showed that the red blood cell level had dropped below $4.0 \times 10^{12}/l$ but improved slightly during the next two infestations.* Other possible conditions e.g. leptospirosis, copper poisoning, were eliminated on the number affected, difference in clinical signs and recovery without treatment. The episode did not occur again. Urinalysis on samples taken later from other calves in the group, for^a/further check on the syndrome, showed epithelial cells, protein, trace glucose, casts and crystals. No quantitation was made on the concentrations of the different constituents found. Leakage of protein/glucose could be attributed to defective filtration/

* N.B. the plasma in the PCV tube was not discoloured.

reabsorption mechanism or direct injury to the nephrons. Formation of casts/crystals is not a sudden change thus the presence suggests a process of injury of some duration. However, further interpretation of these findings would be more meaningful if systematic analyses were drawn from other experimental groups including the uninfested control group, but this was not done. Nonetheless, the urine composition indicated some toxic factor that affected the erythrocytes and renal tissue with concurrent disturbance in cardiovascular function (e.g. subnormal temperature, poor pulse and vasomotor tone). The random samples collected would indicate a renal damage was more frequent than originally thought when the design of the investigations was drawn up.

6.1.6. The musculo-skeletal system manifested muscle wasting and weakness as prominent features and these were confounded by inanition. The animals became thin and showed prominent bone points. Consequently the gait and posture and digestive activity were impaired. In the terminal stages calves became recumbent, unable to rise or, when helped, could not support themselves. In certain cases attempts to extend the flexed head/neck failed as the animals rested in sternal recumbency. Thus this group (III) sustained much more serious effects than group (II) infested with successive low density of adult R. appendiculatus. The nervous system manifested signs associated with pain and irritation

sensations described before (C.6.1) and, in addition grunting and odontopetrisis (grating of teeth simulating mastication) developed terminally. Different reflexes and consciousness were maintained but became feeble terminally. Pruritis, of varied intensity, was commonly induced during the secondary exposures in all calves. The grooming was the same as described for experiment (II) (C.5.1.7) but the calf (T.23) with generalised erythema developed ^{an} exaggerated reaction leading to severe traumatization on several parts of the body including breaking of the horns. Whereas pruritis abated after engorged females had detached, it persisted much longer in this calf. This indicated latent effects from agents injected by the detached female ticks and high individual sensitivity in the calf compared with other members of the group. Thus the response in calf (T.23) presented unique picture not found in the group exposed to successive low density of R. appendiculatus.

6.1.7. Integumentary system was another centre of marked reaction. The direct effects elicited were the same as described in experiment II (C.5.1.8). During the secondary infestations the reactions were confounded by hypersensitivity responses, i.e. bleb formation and urticarial eruptions. The bleb formation developed ^{within} one to two days of infestation accompanied by the same changes e.g. exudation, "scalding" and encrustation. However, disturbance of fresh bleb lesions revealed discrete craters with ^a raw granular base and

light brown edges. Further probing caused easy bleeding and thus, during grooming, the exudate around the lesion was a mixture of blood and plasma fluid that became dark instead of light brown on drying. On healing such craters often left durable 'pox virus' mark scars. Loss of hair in the presence or absence of encrustation was a prominent sign over the ears. The accumulation of exudate, necrotic epidermal tissue and dead ticks in situ emitted a foul smell and attracted flies inside the ears but myiasis was not detected. The flies gained access to the ears before the sleeves were sealed or restored, as this was normally done at the time the engorgement of the female ticks was imminent. Besides, aeration helped in rapid drying of wet surface caused by an inflammatory exudate. Healing progressed under the scabs and hair regrowth lifted the scabs off the area in two to four weeks.

6.1.8. Reappraisal of the clinical signs shows that the successive high density infestations of adult R. appendiculatus caused much more serious effects on the health of the calves than the successive low density infestations of the adult instar. The anaemia, prostration and death could be more closely associated with direct effects of tick infestation in this group (III). Nonetheless, secondary complications, such as abscessation and renal function abnormalities, could produce equally serious consequences. Although the incidence of frank renal signs was low there are

indications that the system can sustain more serious damage than was thought hitherto. Moreover, there is no report in the reviewed literature on renal dysfunction related to tick infestation in the bovine.

6.2. CLINICAL-PATHOLOGICAL FINDINGS

6.2.1. Haematological estimations.

The mean absolute values and the standard deviations of the successive weekly estimations are presented in Figures 6.5 to 6.13. An early and progressive marked decline appeared in the erythrocytic indices, viz: packed cell volumes (PCV), total red blood cell counts (RBC), haemoglobin concentrations (HB) (Figs. 6.5 to 6.7). In contrast, the mean levels of mean corpuscular volumes (MCV) and mean corpuscular haemoglobin concentrations (MCHC) held fairly constant with small deviations throughout, except the MCV, which rose abruptly to levels above normal during the eighth exposure and subsequently dropped to upper level of normal range (Figs. 6.8 & 6.9). The delayed rise in the mean corpuscular volume level indicated critical deterioration accompanied by stimulation of the regenerative haemopoietic potential. The slight recovery in the latter part of the infestations was thus attributed to haemopoietic stimulation and reduced tick burden (Fig. 3.2). The standard deviations were generally small except for the red blood cell counts during the

FIG. 6-5

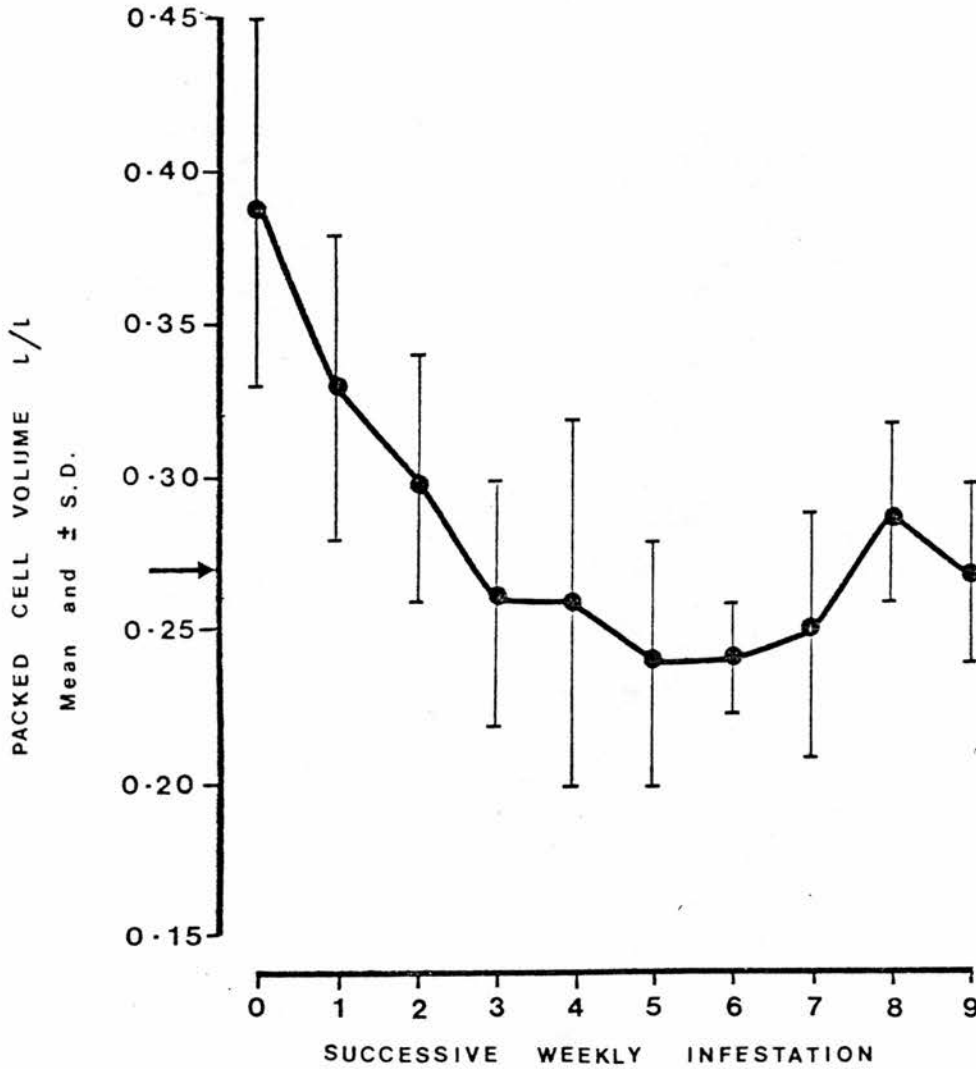


FIG. 6.5

Means and standard deviations of Packed Cell Volumes of five calves exposed weekly to high density infestations of adult Rhipicephalus appendiculatus.

Arrow marks critical level; (n) value dropped to two during last two infestations .

FIG. 6-6

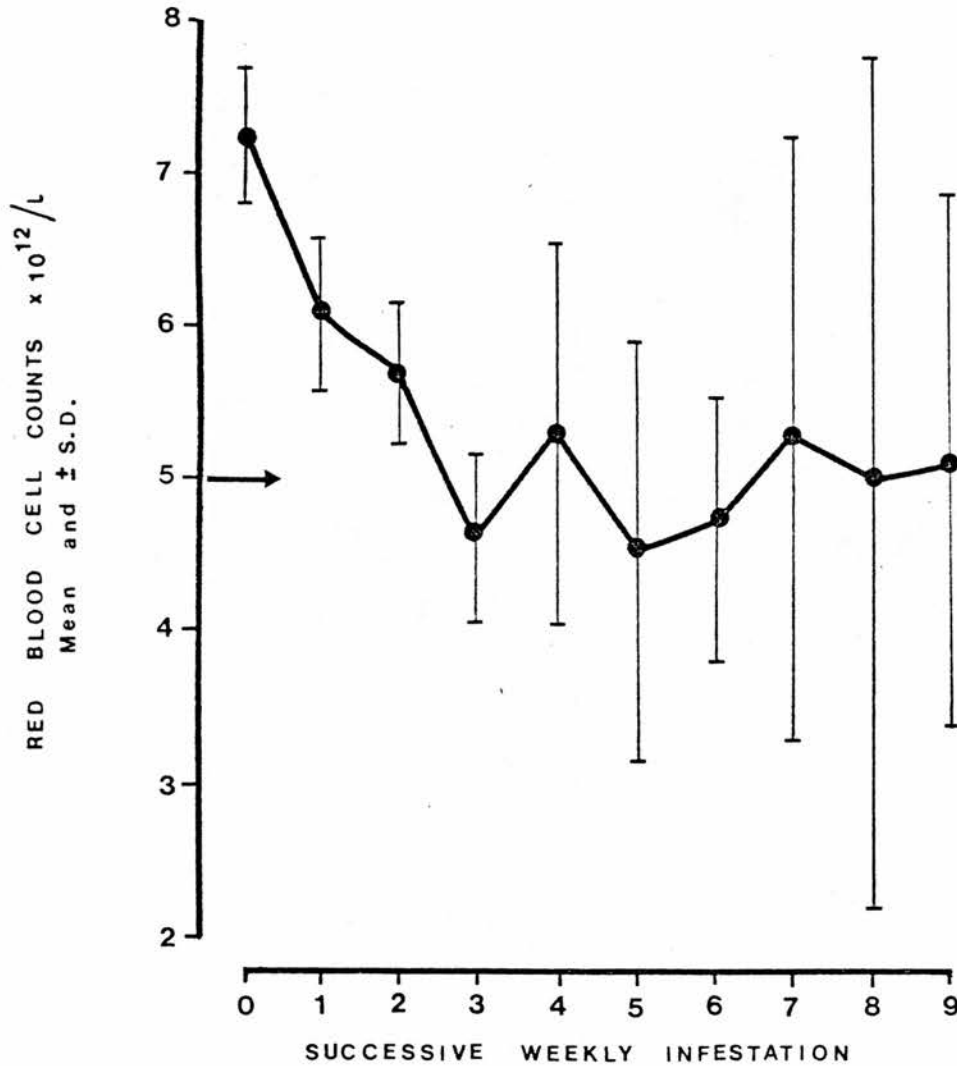


FIG. 6.6

Means and standard deviations of Red Blood Cell counts of five calves exposed weekly to successive high density infestations of adult Rhipicephalus appendiculatus. Arrow marks critical level; (n) value dropped to two during last two infestations .

FIG. 6.7.

Means and standard deviations of Haemoglobin Concentrations of five calves exposed weekly to successive high density infestations of adult Rhipicephalus appendiculatus. (n) value dropped to two during last two infestations.

FIG. 6.8.

Means and standard deviations of Mean Corpuscular Volumes of five calves exposed weekly to successive high density infestations of adult Rhipicephalus appendiculatus. (n) value dropped to two during last infestations.

FIG. 6-7

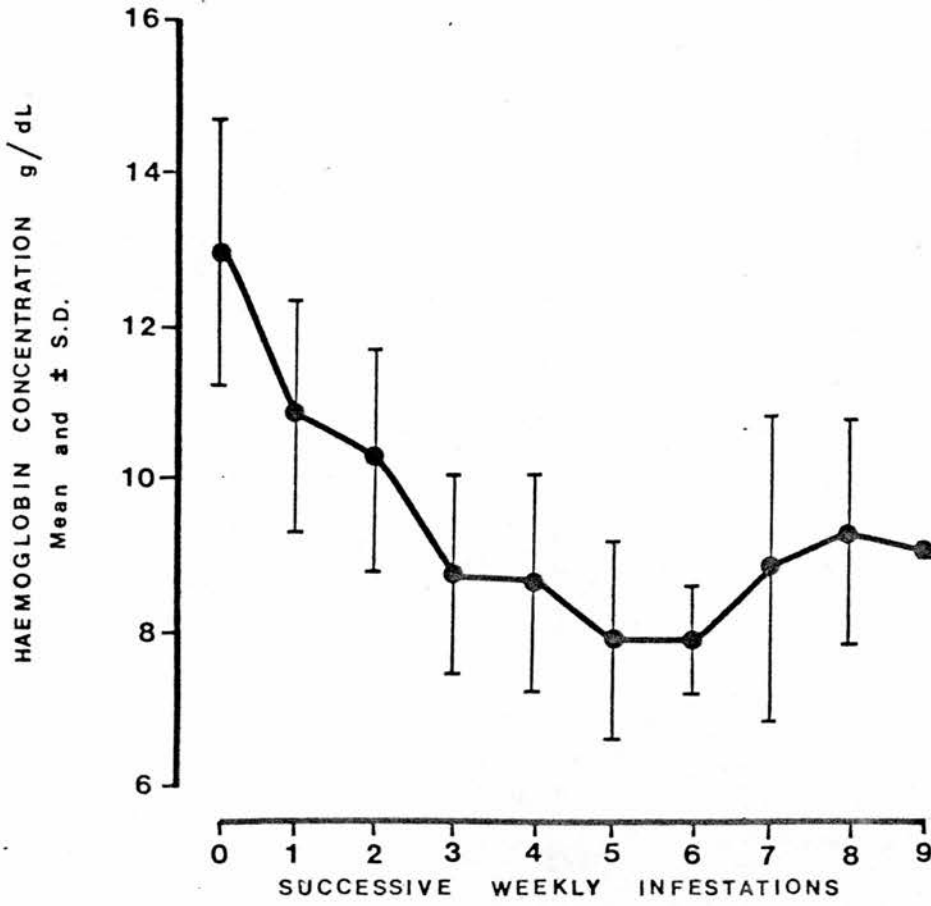
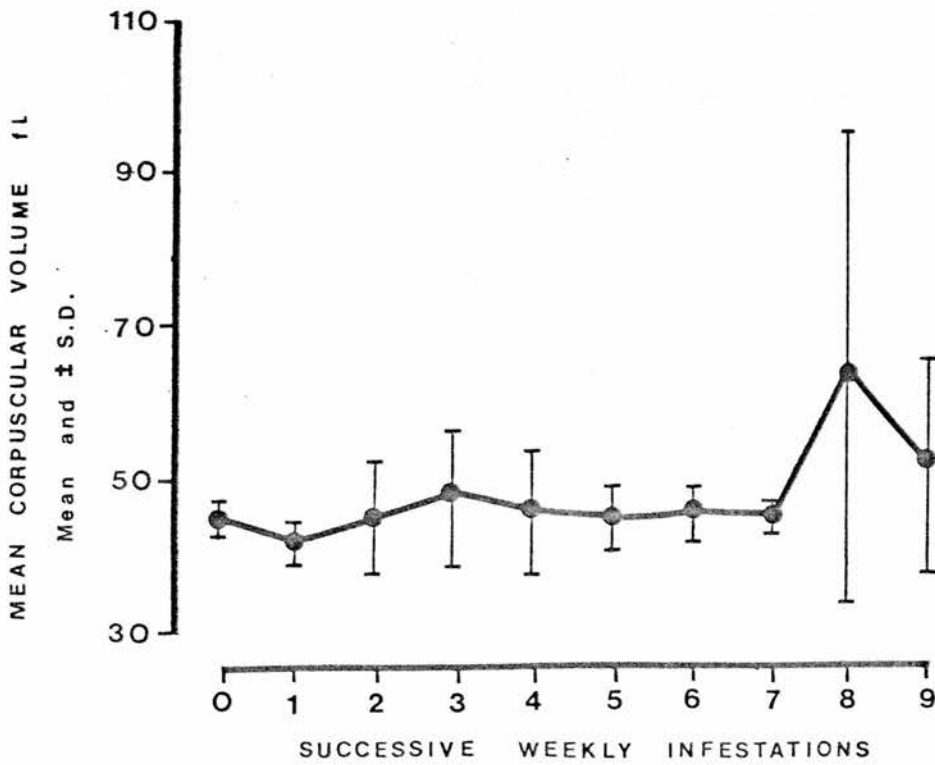


FIG. 6-8



fourth week onwards and the mean corpuscular volumes in the last two weeks. However, the standard deviation for the packed cell volumes, whilst moderately big initially, decreased subsequently. The regenerative features appeared at a crisis period, embracing the fifth week onwards, and were represented by polychromasia, anisocytosis, poikilocytosis, reticulocytosis (2 to 3%) and transient increase in MCV index (macrocytic). Thus the net response was a 'macrocytic' normochromic anaemia. Deaths appeared at the crisis time. However, the calf (T.22) that survived did steadily recover commencing at the eighth exposure and attained normal levels shortly after termination of infestations. Thus the major contribution to the mean regenerative responses illustrated (Figs. 6.5 to 6.8) was attributable to this calf as by the seventh infestation only two calves were standing (abscessed one not counted). The haematological recovery showed ^{that} the regime of infestation applied did not produce aplastic anaemia as had been suggested by van Rensburg (1959). The means of the absolute values of the leucocytic series were within normal ranges throughout the exposure, but slight leucocytosis and neutrophilia associated with abscessation appeared at the sixth exposure (Figs. 6.10 to 6.13). The levels of eosinophils were low and the cells were found in 20% only of the estimations and were not detected after the fifth infestation. The disappearance could be associated with stress from effects of the infestations (Schalm

FIG.6.9.

Means and standard deviations of Mean Corpuscular Haemoglobin Concentrations of five calves exposed weekly to successive high density infestations of Rhipicephalus appendiculatus. (n) value dropped to two during last two infestations.

FIG. 6.10.

Means and standard deviations of White Blood Cell counts of five calves exposed weekly to successive high density infestations of adult Rhipicephalus appendiculatus. (n) value dropped to two during last two infestations.

FIG. 6-9

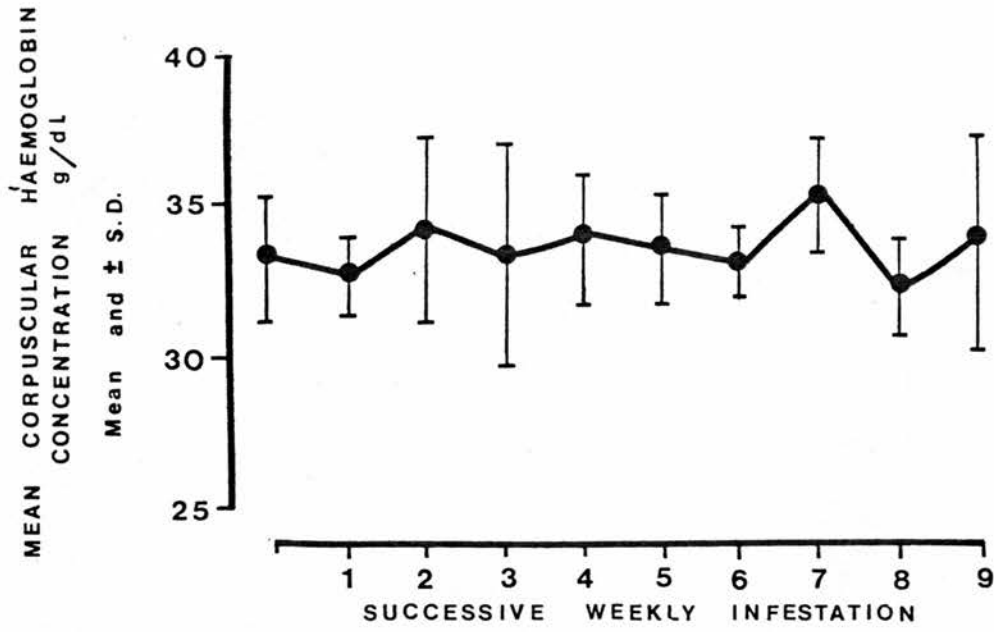


FIG. 6.10

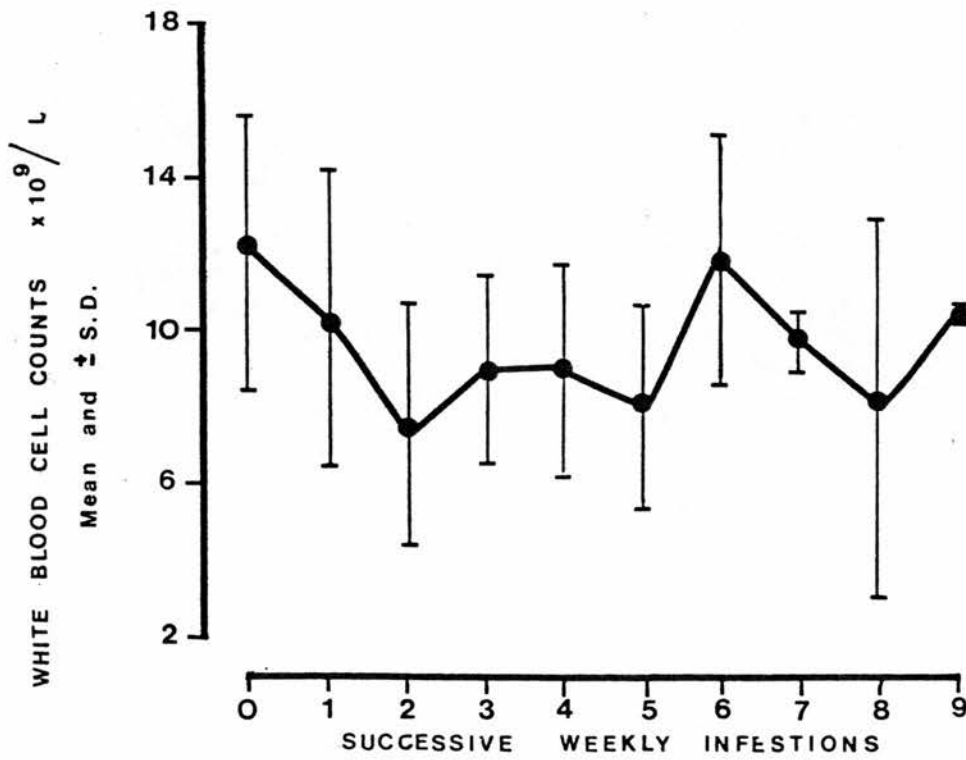


FIG. 6.11

Means and standard deviations of absolute Neutrophil Cell counts of five calves exposed weekly to successive high density infestations of adult Rhipicephalus appendiculatus. (n) value dropped to two during last two infestations .

FIG. 6.12

Means and standard deviations of absolute Lymphocyte Cell counts of five calves exposed weekly to successive high density infestations of adult Rhipicephalus appendiculatus. (n) value dropped to two during last two infestations .

FIG. 6-11

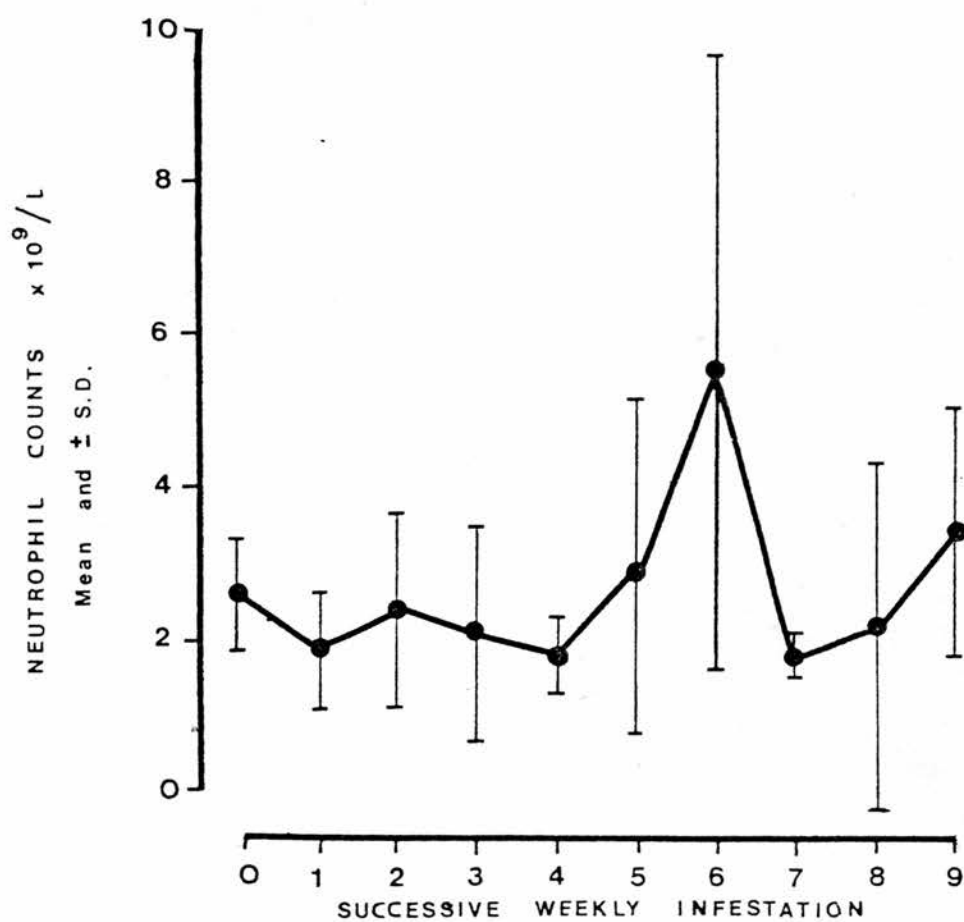


FIG. 6-12

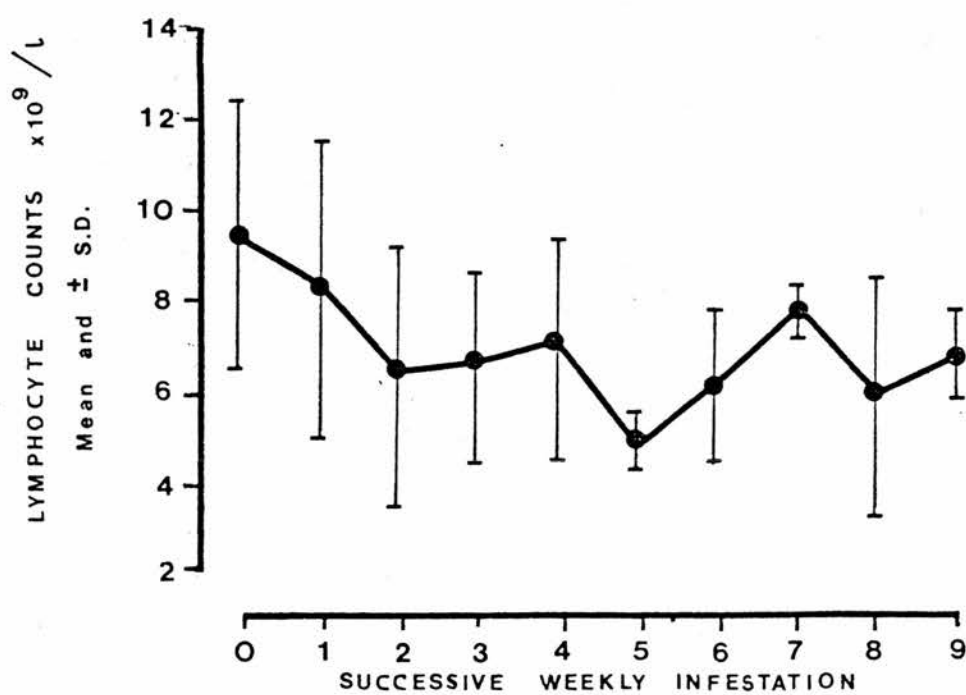


FIG. 6-13

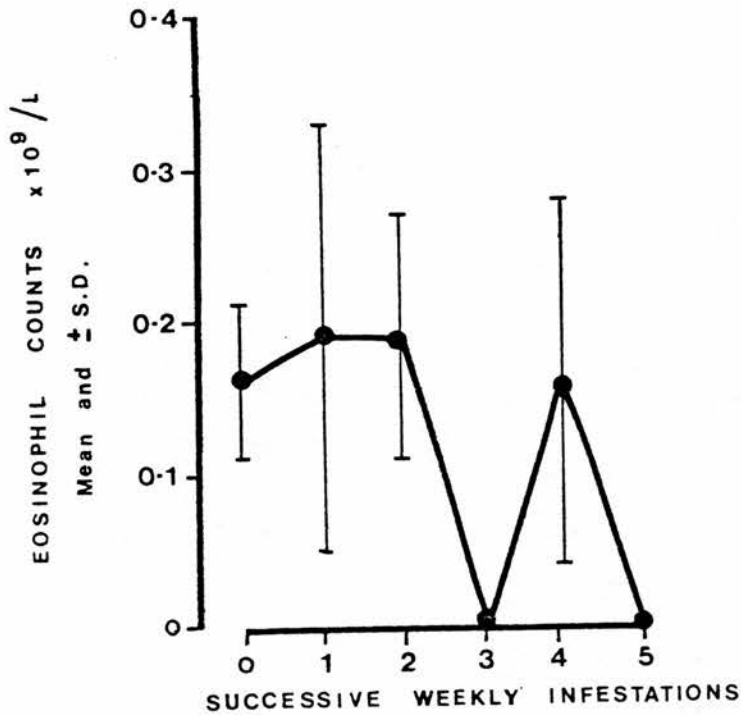


FIG. 6.13.

Means and standard deviations of absolute Eosinophil Cell counts of five calves exposed weekly to successive high density infestations of adult Rhipicephalus appendiculatus. (n) value dropped to two during last two infestations.

et al., (1975). The standard deviations were similarly small except during the sixth week (Fig. 6.11).

6.2.2. Analysis of variance, as described before, revealed significant differences between the uninfested control group (I) and other experimental groups, including this group (III), in all estimated parameters (Tables 4.1 to 4.9), except neutrophils (Table 4.7).

Duncan's New Multiple Range test (DNMRT) resolved significant subsets in which the group (III) responded independently and similarly to other experimental groups and overlapped between subsets (Tables 4.10 to 4.17). The test ranked the group as follows. Lowest values ranking at greater than 50% of the analyses showing significant variance were attained in each of these indices, packed cell volumes, red blood cell counts, haemoglobin concentrations (Tables 4.10 to 4.12). Similarly the lowest values at less than 50% of the same analyses were attained in each of ^{the} /mean corpuscular volumes (MCV) and absolute eosinophil counts (Tables 4.13 & 4.17). Conversely the highest values ranking at less than 50% of the same analyses were attained in each of MCV, absolute eosinophil counts and total white blood cell counts (WBC) (Table 4.15). In other rankings the group assumed an intermediate position in the subsets with lowest or highest values (Tables 4.11, 4.12, 4.14; 4.13, 4.14 & 4.16). Mid-way position ranking designated 'overlap between subsets' is illustrated in Tables 4.10, 4.12, 4.13, 4.15 to 4.17. Thus the test

revealed the lowest red blood cell counts matched the results of group (VI) (infested with concurrent three instars of R. appendiculatus) which was the second experimental group to sustain severe effects. Overall the group showed closest association with groups (IV) (infested with nymphs) and (V) (infested with larvae), since estimations for group (VI) stopped after only three infestations. This association emphasizes the difference between this group (III) and group (II) which is related to the densities and intervals of infestations. The lowest ranking in the packed cell volumes, red blood cell counts and haemoglobin concentrations further supports the diagnosis of severe anaemia induced under this infestation regime relative to other regimes.

6.2.3. Biochemical estimations

The mean values and standard deviations of serum levels of protein (S.P.), immunoglobulins (IG) and glucose (S.G.) are presented in Figures 6.14 to 6.16. The mean levels of S.P. and IG showed a definite decline, culminating in marginal and subnormal levels for serum protein. The mean values of immunoglobulins remained above the minimal critical level (20 turbidity units). The missing and incomplete data (Figs. 6.15, 6.16) arose from technical difficulties. The trend in the responses of the three parameters showed no recovery to initial levels in later stages of exposure which concurs with the haematological responses. Thus, the

decline in the serum protein levels corresponded with that of erythrocytic indices, but the decline was less steep. This indicates that the S.P. was less sensitive to the tick feeding effects. The mean values for S.G. were within normal range and showed a slight fluctuation. However, a definite assessment cannot be made because estimations were not continued through the entire experiment. The standard deviations of the three parameters were generally small, being compact for serum glucose but with some greater ranges in the serum protein levels. The cause of the greater fluctuations is not clear as there was no corresponding change in the immunoglobulin levels and the possibility of haemoconcentration in individual calves at these times was unlikely as no indications were revealed on inspection of other clinical parameters. The low mean levels of serum protein could partly explain the dropsical changes revealed at autopsy. The deterioration in serum protein levels toward hypoproteinaemia could be attributed to blood loss, reduced feed intake and wasting in body tissues. The decline in the immunoglobulin levels and absence of significant variance between groups in the mean values during infestations indicates suppression of the immunoglobulin forming system thus presenting similar levels to those of the uninfested control group.

FIG. 6.14.

Means and standard deviations of Serum Protein levels of five calves exposed weekly to successive high density infestations of adult Rhipicephalus appendiculatus. Arrow marks normal critical level; (n) value dropped to two during last two infestations.

FIG. 6.15.

Means and standard deviations of Immunoglobulin levels of five calves exposed weekly to successive high density infestations of Rhipicephalus appendiculatus. Arrow marks safe level; (n) value dropped to two during last two infestations).

FIG. 6-14

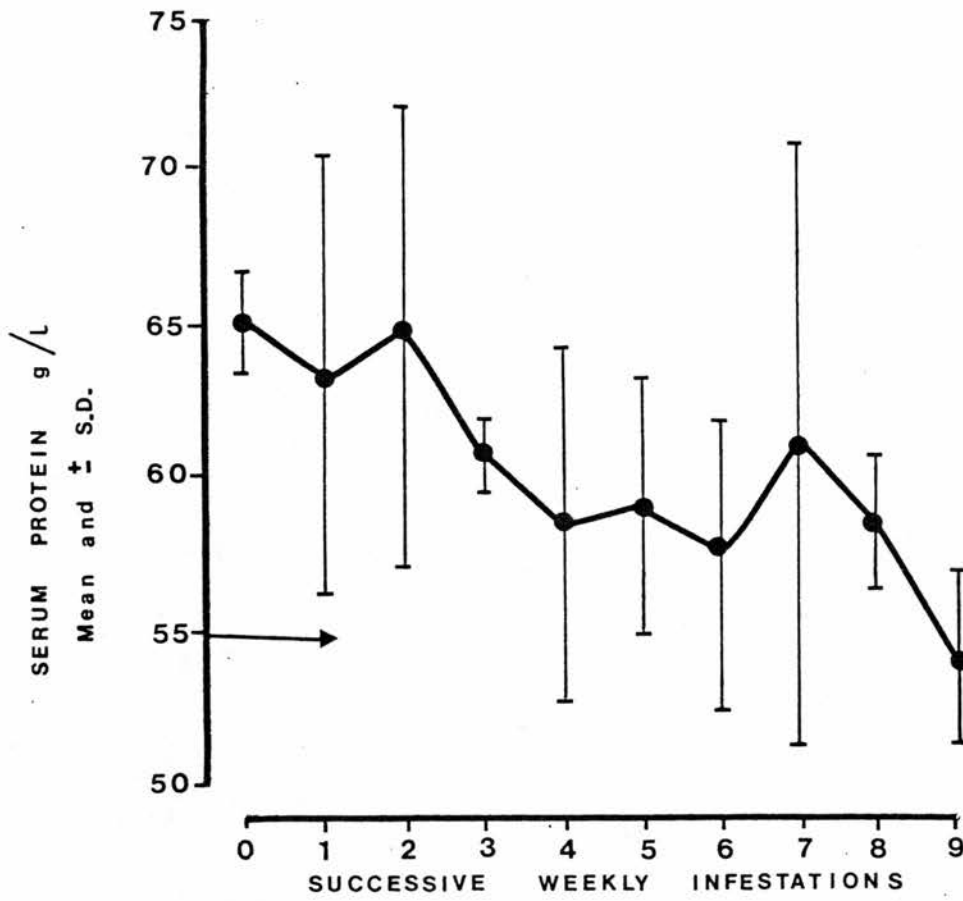


FIG. 6-15

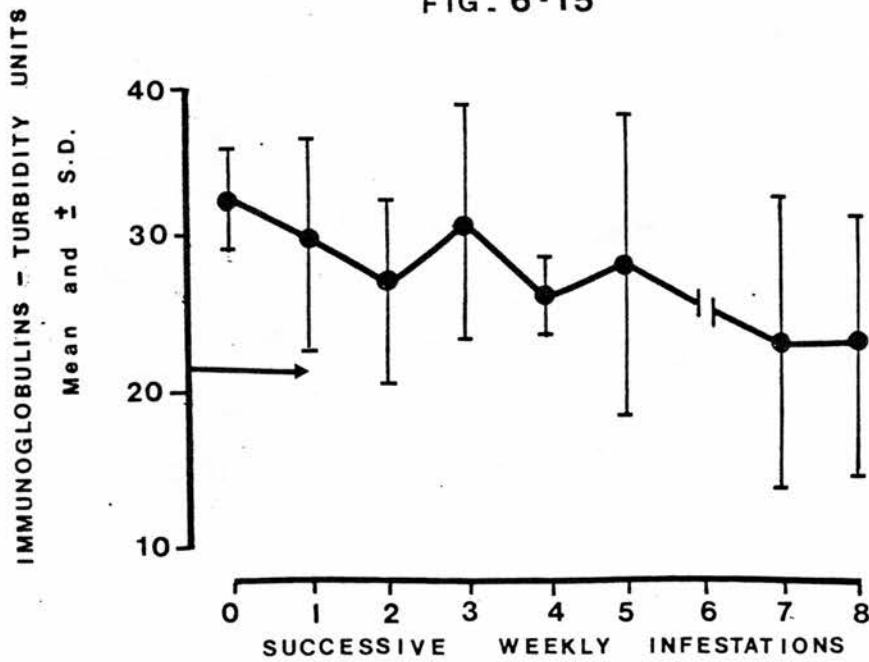


FIG. 6-16

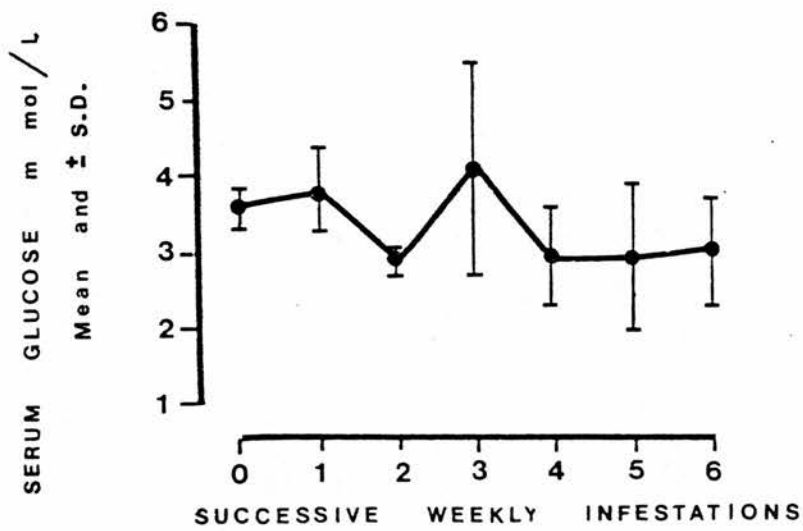


FIG. 6.16

Means and standard deviations of Serum Glucose levels of five calves exposed weekly to successive high density infestations of Rhipicephalus appendiculatus.

6.2.4. Similarly analysis of variance revealed significant differences between the uninfested control group and other experimental groups, including this group (III), in serum protein and serum glucose levels during infestations (Tables 4.18, 4.20). The significance was found at both 1% and 5% levels. However, the significant differences in the immunoglobulin levels during the pre-infestation analyses disappeared with infestations.

The Duncan's New Multiple Range test resolved significant subsets in which the group (III) responded similarly to other groups and overlapped between subsets during infestations (Tables 4.21, 4.23). Thus the group (III) ranked lowest on one occasion in each of serum glucose and serum protein and once assumed the highest rank in the serum glucose during the first exposure. Overall, the test associated the group closest to groups (I) (uninfested controls), (IV) (infested with nymphs) and (V) (infested with larvae) followed by group (II) (low density adults). The few times the significant differences were revealed during the infestations indicates the parameters showed similar responses in general to tick effects and maintained levels close to the controls. Thus in addition to lack of variance in immunoglobulin levels during infestations it can be suggested the three parameters were less sensitive indicators for specific tick effects.

6.3. AUTOPSIES

6.3.1. Three natural deaths occurred between the sixth and tenth infestations and two, the survivor (T.22) and the abscessed (T.27) were slaughtered a month and a week respectively after termination of the experiment. The gross pathological changes were as follows. Carcass condition varied, being good (T.22), fair (T.27), and emaciated to cachexic with sunken eyes in the rest. The integument showed attached ticks, tarnished rough coat, alopecia, abrasions, encrustations (4 calves), pityriasis (3 calves), hyperkeratosis (2 calves), gross thickening of pinnae (3 calves) and distortion of conchal cartilage (one calf). Mucosae colour ranged from pink to congested (3 calves) and pale (2 calves). The labial and gingival vessels were prominent in one calf and thick ocular discharge appeared in one calf. Subcutis devoid of fat revealed wasted muscle (dominated by white striations) and dropsical changes in the connective tissue (presenting wet surface). Lymph nodes showed slightly to moderately enlarged head nodes (4 calves), oedematous and haemorrhagic changes in local drainage nodes (3 calves) and abscessed parotid node (one calf). Haemolymph nodes were moderately enlarged in one calf.

6.3.2. Abdomen. Ascites was revealed in one calf. Spleen(s) were firm in texture and shrivelled, and the cut surface was dominated by white pulp (4 calves); probably because the red pulp was reduced in amount. Other lesions found were capsular petechiae (one calf)

and small abscess (one calf). Liver(s) appeared red/congested with thickened edges (3 calves). Gall bladders showed no abnormality. Kidneys showed congestion and gelatinous infiltration in calyces/pelvis (3 calves). Adrenal glands were small to moderate in size and congested (3 calves). The gastro-intestinal tract lacked mesenteric fat (one calf), otherwise others showed scanty fat. The rumen was moderately full (4 calves), with congested serosal and submucosal areas (one calf). The abomasum showed mucosal congestion/haemorrhages (2 calves), superficial ulceration and red/brown discolouration of the mucosa (2 calves), and oedema of the lamellae (2 calves). The small intestine was congested (3 calves) and the peyer's patches eroded (one calf). Caeca were congested with prominent serosal vessels (3 calves) and rectum congested in one calf.

6.3.3. Thorax. One calf showed hydrothorax. The heart carried multiple changes: hydropericardium (3 calves) with fibrin strands (one calf), coronary fat lacking or degenerate (gelatinous) and prominent coronary vessels (3 calves), epicardial white striations and petechiae (one calf each), myocardial congestion (one calf) and flabby right ventricle (one calf), and endocarditis in one calf (haemorrhagic and eroded cuspid valves). Lungs showed marked generalised congestion of the parenchyma and passages (3 calves), consolidation and bronchiolar thick brown-red exudate (T.27 - with abscessed parotid

node) and froth in bronchioles (terminal, one calf). Thus calf (T.27) developed identical lymph node-pulmonary changes associated with abscessation as developed in calf (T.5) (which died of chronic bronchopneumonia (C.5.3)). The appearance of the bone marrow of the femur and humerus indicated some activity, and showed uniformly red firm pulp, or red-brown or sanguineous gelatinous consistency or haemorrhagic changes. The persistence of the activity was demonstrated by the steady clinical response in the calf (T.22) that survived.

6.3.4. Conclusions. The congestion and haemorrhagic reactions in tissues could be associated partly with terminal septicaemia. Nevertheless, the vascular changes in local drainage lymph nodes were a direct result of exposure to products drained from the infested areas. These drained products invariably contained tick salivary secretions. The transudate and other dropsical changes were associated with anaemia, hypoproteinaemia, inanition, cachexia and probable effects of toxic agents on the circulatory endothelium. The pneumonic changes had a direct link with the parotid node abscessation although the link with the generalised congestion of the parenchyma in other calves could not be established. The bone marrow appearance indicated persistence of haemopoietic activity. The shrinkage of the spleen was spectacular but the pathogenesis is not clear, though it can be attributed to the general tissue wasting. Judging the blood picture levels at death,

anaemia could be discounted as being the primary cause of death in certain cases. The persistence of digestive system function (moderately filled rumen) would eliminate inanition as/^apredominant cause of emaciation and extreme weakness connected with prostration terminally. Thus it can be suggested that the progressive deterioration in clinical condition owes a lot to the direct effects of tick feeding as revealed also by the responses to successive low density infestation of adult R. appendiculatus (C.5.1.2). The direct effects could be associated with intoxication from protracted tick feeding, especially the females which can secrete copious saliva during engorgement phases. The higher death percentage under this infestation regime shows the density factor was a major function in the eventual effects of tick feeding on the health of the calves. Nonetheless, secondary effects, e.g. bacterial pneumonia, should always be considered seriously.

6.4. SUMMARY OF SIGNIFICANT FINDINGS

Weekly high density infestations of adult Rhipicephalus appendiculatus severely affected the health of the calves and caused 80% loss through secondary infection and death from tick effects. The responses shown by individual calves varied in severity and are presented by the following manifestations. 1) Immediate reaction

to attachment aroused pain and irritation sensations predominantly during secondary exposures. 2) Loss of body condition culminated in emaciation and cachexia. 3) Development of extreme depression and weakness followed by prostration prior to death were common. 4) Varied pyrexia levels manifested as mono and diphasic patterns were induced but they regressed during later exposures to normal. Terminally the fatal cases showed subnormal rectal temperatures. 5) Significant disturbance in other cardiovascular system parameters induced durable tachycardia, congestion or pallor and excessive secretions/ discharges in visible mucosae, cutaneous erythema and cyanosis and oedema in cutaneous and subcutaneous tissues. 6) Lymphadenopathy of declining magnitude complicated by abscessation in regional drainage nodes during infestations was constantly produced. 7) Respiratory derangement led to cough, tachypnoea and nasal discharges. 8) Digestive system activity persisted despite drastic reduction in feed intake associated with critical deterioration in body condition terminally. 9) Complication of haemoglobinuria of an unestablished origin appeared during secondary exposure; but haematological investigations revealed no blood-borne parasites. 10) Pruritis was constantly provoked during secondary infestations. 11) The direct effects of tick feeding caused exudation, eczema, alopecia and pinna distortion; and the indirect effects induced a hypersensitivity state that was associated with bleb formation and urticarial eruptions.

12) Disturbance of blood composition induced marked anaemic changes, transient leucocytosis and neutrophilia, suboptimal serum protein levels. Significant statistical differences between the group and other experimental groups, including the uninfested controls, were revealed in all haematological/biochemical parameters estimated except one. 13) Autopsies revealed morbid tissue changes attributable to direct tick effects and resultant complications, e.g. terminal septicaemia, suppurative reactions. Severe tissue wasting and integumentary lesions were prominent. Mucosae, serosae and organ parenchyma (lymph node) showed one or a combination of these changes, viz: haemorrhages, congestion and pallor. Dropsical changes were found in serous cavities and around lymph nodes. Serous atrophy of fat of the coronary grooves, subcutis and renal calyces/pelvis occurred. Broncho-pneumonic changes associated with lymph node abscessation (parotid) and recovery of Corynebacterium bovis from both sites were revealed. The spleen was markedly reduced in size in the majority of cases. Bone marrow activity persisted despite the severe clinical reaction.

Thus the clinical-pathological responses manifested could be attributed to both direct tick effects and resultant secondary complications. However, death could not be specifically attributed to either, but both appeared to work in consort.

CHAPTER 7

EXPERIMENT (IV): RESPONSES OF FIVE CALVES EXPOSED WEEKLY
TO SUCCESSIVE INFESTATIONS OF NYMPHAL RHIPICEPHALUS
APPENDICULATUS

All calves aged five to eight months and not previously exposed to tick effects were in good health at the start of the experimental infestation. The nymphs were applied weekly on individual calves at varied densities during the successive infestations. The mean numbers of the unfed nymphs applied and the engorged recovered during successive infestations are presented in Figure 3.3. The calves reacted to the infestations but the effects, especially the systemic, were generally moderate. Thus the manifestations induced are described as for previous experiments.

7.1. PHYSICAL (CLINICAL) SIGNS

7.1.1. The recorded clinical signs were derived by regular observation and physical examination of the different systems. The calves were restrained to limit grooming that would cause disruption of tick performance but were released after all engorged ticks had detached. Immediate reaction to attachment, as observed in other experiments (C.5.1, C.6.1), was manifested by struggling; frantic head/ear shaking; excessive secretions e.g. frothy saliva, muzzle sweating, lachrymation; frequent urination; and refusal to feed for hours. The signs

were induced once or several times in individual calves throughout the experiment and were transitory. However, during secondary infestations some of the signs, e.g. head/ear shaking, frequent urination, recurred at en-^{the}gorgement or persisted through/infestation period, e.g. excessive secretions and irritation sensation. Observations on various systems revealed the following reactions.

7.1.2. A gradual and moderate loss in body condition occurred and was judged by the prominence of bones through muscle wasting, hollowness of paralumbar fossae and appearance of eyes and coat. The deterioration was more marked during periods of intense irritation when animals became restless and unable to feed continuously. Generally the calves showed bright and active demeanour and physique throughout, except one calf became dull and weak during the later stages of the experiment. The reaction over the ears was quite marked during most exposures and this could partly explain the change in disposition.

7.1.3. The cardiovascular system was less severely affected compared with those exposed to low and high density infestations of adult Rhipicephalus appendiculatus ticks. Pyrexia was generally manifested by mild and transient elevation of mean rectal temperatures during the successive infestations as illustrated in Figure 7.1. The slightly

FIG. 7.1

Mean weekly Rectal Temperatures of five calves exposed weekly to successive nymphal infestations of Rhipicephalus appendiculatus compared with uninfested controls. (Arrow marks normal critical temperature).

FIG. 7.2

Mean daily Rectal Temperatures of five calves exposed to fourth successive weekly nymphal infestation of Rhipicephalus appendiculatus compared with uninfested controls. (Arrow marks normal critical temperature).

FIG. 7-1

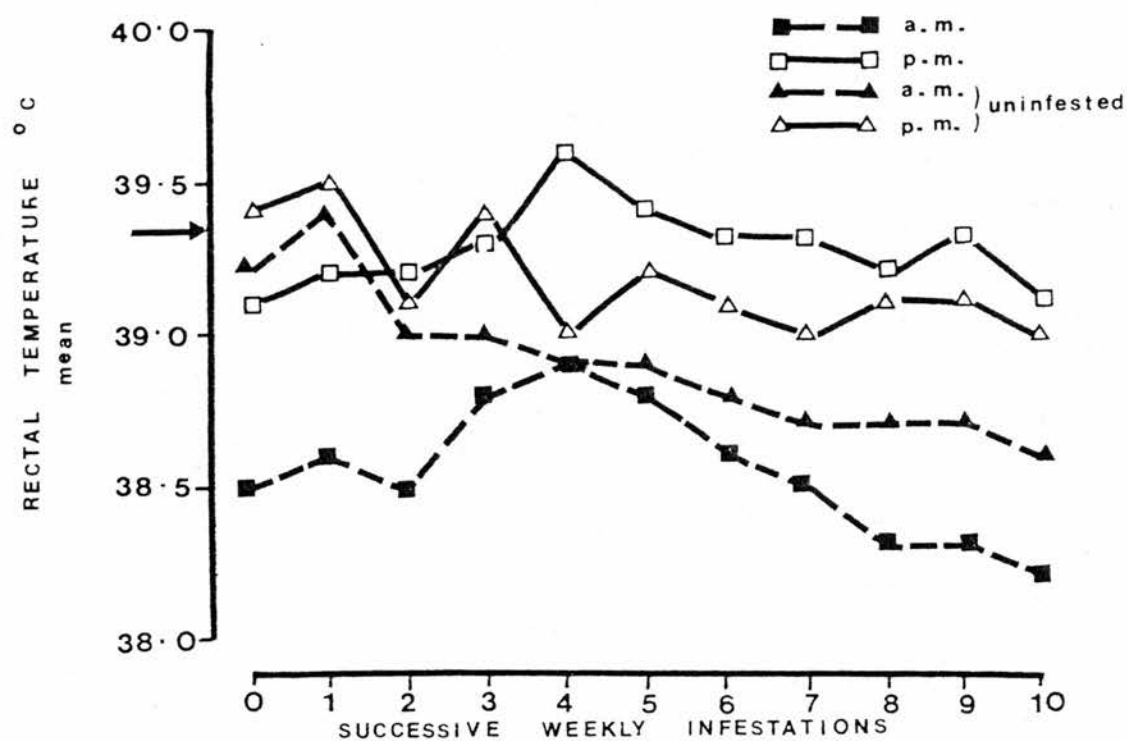
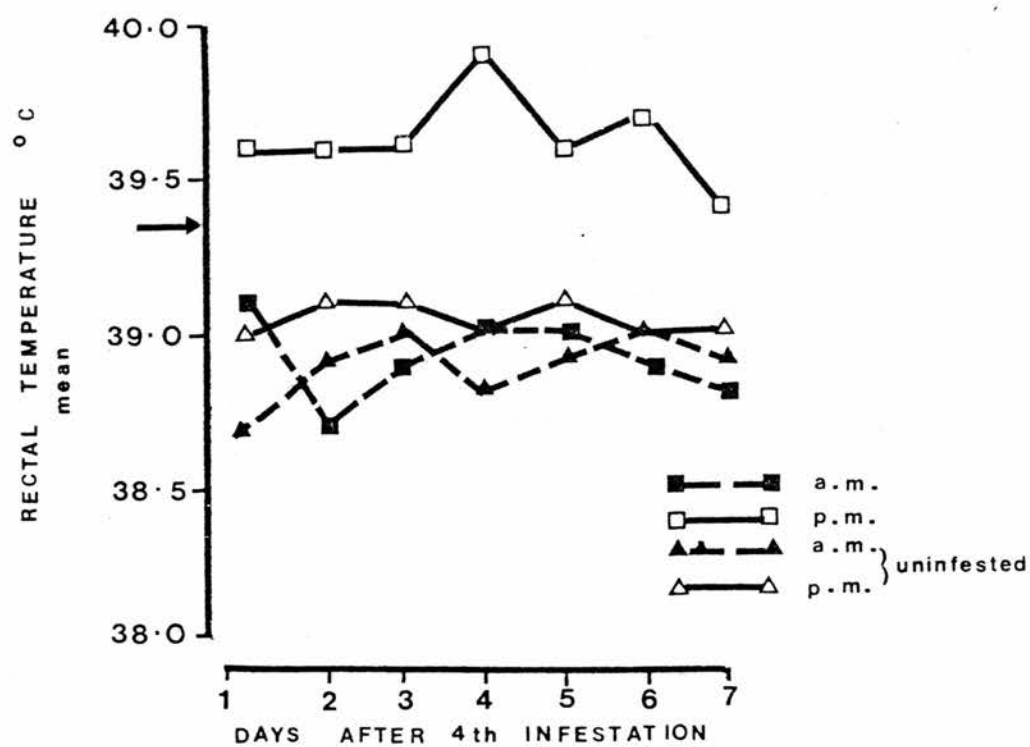


FIG. 7-2



elevated mean rectal temperatures shown by the compared uninfested control group (I) was due to non-specific reactions. However, the mean levels of the p.m. temperatures for both, the group (IV) and the controls (I) closely matched though after the fourth exposure this group (IV) assumed the upper position in the high normal range close to the critical temperature (39.3°C). The single highest mean weekly pyrexia peak at the fourth infestation is amplified in Fig. 7.2. The amplification demonstrates variation in the mean daily rectal temperatures. It is noteworthy that the mean daily rectal temperatures (p.m.) were elevated throughout and the peak coincided with the engorgement of the nymphs. Thus this response markedly contrasted with the reaction of the compared uninfested control group. The pyrexia levels were nonetheless in the slight range. The clear distinction shown between the group and the controls indicates the responses were not induced by non-specific factors as could be deduced from Figure 7.1. Another salient feature revealed in the amplification is the immediate pyrexia on infestation, a response which was also exhibited in other infestation regimes (C.5.1., C.6.1) during secondary exposures. The immediate pyrexia indicates a feature of ^a/hypersensitivity state. A steady reversal toward the normal temperatures (p.m.) tended to follow completion of feeding and detachment of the engorged nymphs. This further illustrates that the pyrexia was caused by tick secretion during feeding and not an infectious agent transmissible by the tick. Heart and pulse

characteristics showed little or no significant derangement and where detected coincided with engorgement of the nymphs. At that stage the pulse rates and force of the heart and pulse qualities increased but were reversed soon after repletion of the tick was completed. The mucous membranes occasionally showed congestion of the conjunctivae on the side(s) corresponding with the infested ear(s). Excessive secretions from the mucosae frequently appeared at different times in all calves and were represented by lachrymation, predominantly mucoid nasal discharge, and copious salivary secretion. The secretion of copious saliva for no apparent reason was observed in two calves. It is interesting to note that a similar reaction was provoked by infestations of adult Rhipicephalus appendiculatus. Erythema of a mild nature appeared round infested areas and was very seldomly detected in the contiguous or remote unpigmented skin. Mild oedema developed around the same sites, but oedema of a more intense degree was detected twice round the sub-parotid and intermandibular space in two calves. However, oedema observed beyond the infested pinna was temporary and was almost undetectable by the second day.

7.1.4. Lymphadenopathy of a low grade was induced in all calves and affected the parotid nodes. The average maximum size produced was two to three times the original estimation. The discernible enlargement developed rather slowly but the regression was rapid after all the ticks had dropped. The response to the nymphal effects was

much less intense than that induced by the infestations of adult R . appendiculatus. Thus the course of lymph node reaction between these infestation regimes (C.5.1, C.6.1 & C.7.1) differed in onset, duration and in the development of detected complications. In the earlier regimes onset was more rapid and duration longer and were accompanied by complications e.g. abscessation. The respiratory system showed no significant abnormalities throughout except the frequent predominantly mucoid nasal discharges produced by all calves at different times. The discharge could have been increased by draining excessive ocular secretions through the nasolachrymal duct.

7.1.5. Digestive system showed no significant disturbance in function, except for the temporary interruption in feeding at the beginning of infestations and the profuse salivation. The cause of profuse salivation remained unexplained because careful and detailed examination of the mouth, pharyngeal region and other related parts of the head revealed no apparent lesions. The coincidence with the active phase of feeding of the ticks causing irritation sensation could indicate a stimulus of nervous origin. The urinary system remained clinically normal and the urine voided appeared visibly unaffected in colour, clarity and odour and the few random samples tested with labstix ^{acetest} /revealed normal pH, no protein nor ketones. The samples served only as a screening measure in anticipation of the urine changes

observed in experiment (III) (C.6.1.5.). However, the frequent urination developed at various infestations was associated with pain and irritation from the infested ears. The nervous system reaction was dominated by signs associated with pain and irritability during feeding of the nymphs. Irritation resulted in varied degrees of pruritis predominantly during secondary infestations. Animals, when not securely restrained, groomed by rubbing against objects or ^{by}/pawing (scratching) which caused skin abrasions and also loss of some ticks. It was also observed that the calves considerably licked the trunk where ticks were not applied which was suggestive of widely based irritability. Secure restraint, restricting effective grooming, made animals restless, stamp the ground, extend and shake the head/ears and jerk themselves against the restraint devices. Irrespective of the degree of restraint the shaking of the head or ears was constant behaviour. Failure to relieve the irritation through restraint was associated with generation of increased warmth and moisture (sweating) over the general surface of the body during several infestations in two calves. The reaction over the body was very easily perceived even by light stroking of the coat. The urge to groom intensified during engorgement and rapidly abated after all engorged nymphs had dropped.

7.1.6. Integumentary system presented other signs in addition to those described under cardiovascular changes (C.7.1.3.). The primary effects were related to tick bite wounds which produced varied amount of exudate that formed encrustations on drying. The exudation from the feed wounds was augmented during secondary infestations. The affected ears were thickly encrusted and the scabs left hairless areas on lifting. In some cases prominent scars were found, especially during secondary infestations. Nevertheless, the pinna retained its shape. Healing of lesions and hair regrowth took two to three weeks allowing for abrasions inflicted through pruritis. During secondary infestations mild bleb formation of varied frequency was induced in all animals. The blebs were accompanied by increased exudation that trapped some of the nymphs on coagulation. The sticky nature of the exudate indicated high protein content. The blebs were predominantly of the papule/pustule type and appeared within two to three days of the infestation. They were aggravated by vigorous grooming. The scarcity of pseudovesicle type of blebs and the timing of onset shows a distinction from ^{the} responses induced in other infestation regimes (C.5.1., C.6.1. & C.8.1.) where common pseudovesicles and earlier onset were observed. This difference could partly be related to mode of feeding and antigenicity of the secretions of the nymph at the densities applied. Density could be an important factor as the

severe reaction in larval infestations were commonly associated with applications above an estimated 30 000 larvae per exposure. Another feature undetected was the development of urticarial eruptions but there is a possibility they were missed on account of the mild nature of the hypersensitivity reactions.

7.2. CLINICAL-PATHOLOGICAL FINDINGS

7.2.1. Haematological estimations.

The means and standard deviations of absolute values from the various parameters estimated are illustrated in Figs. 7.3 to 7.11. Estimations for the tenth week were not done for technical reasons. The mean values of the packed cell volumes (PCV) were within normal range but terminally declined toward subnormal levels (Fig. 7.3). The decline was gentle with ^atransient small rise round the fifth to seventh infestations. Thus the original levels were not recovered. The standard deviations were consistently small throughout. The mean values of red blood cell counts (RBC) and haemoglobin concentrations (HB) presented corresponding trends but the standard deviations were bigger (Figs. 7.4 & 7.5). However, the correlation between the RBC and the other two indices (PCV, HB) was significant ($r = 0.588, 0.88; p < 0.001$). The mean levels of mean corpuscular volumes (MCV) (Fig. 7.6) were within normal range. However, there was early

FIG. 7.3

Means and standard deviations of Packed Cell
Volumes of five calves exposed weekly to successive
nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7.4

Means and standard deviations of Red Blood Cell
counts of five calves exposed weekly to successive
nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7-3

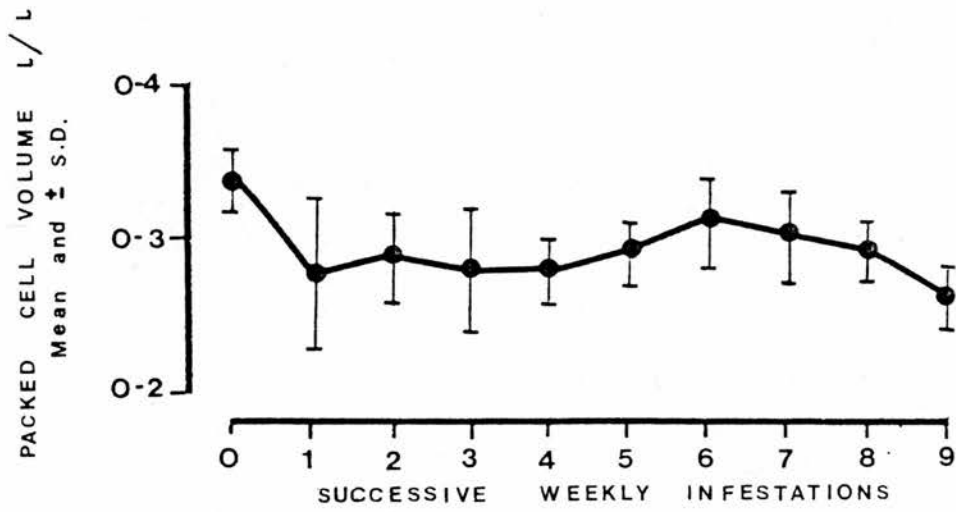
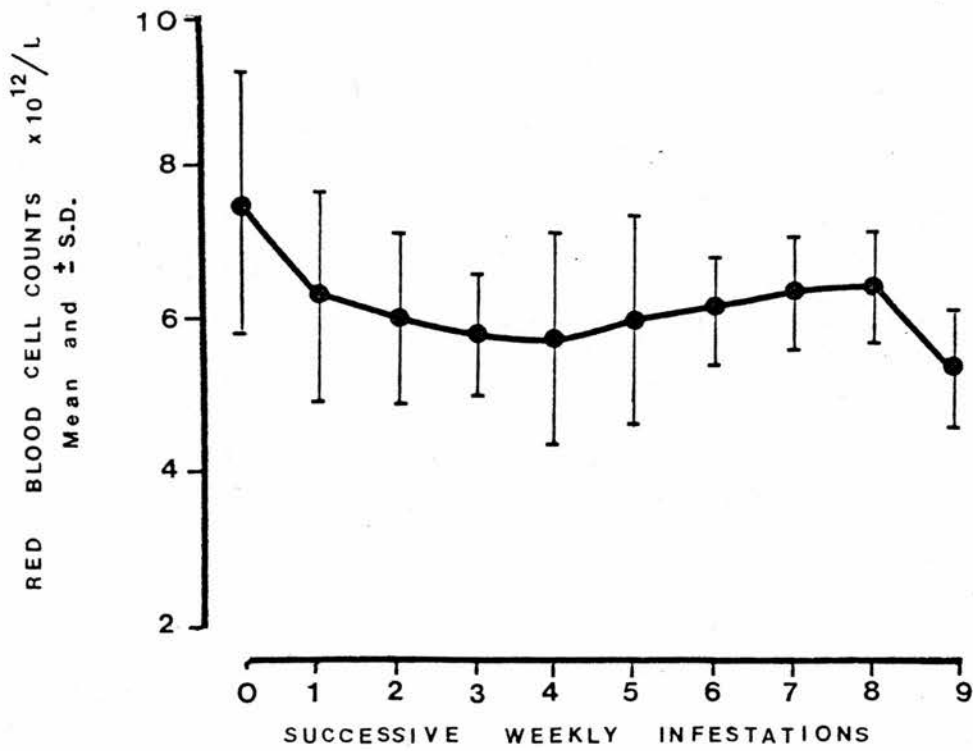


FIG. 7-4



indication of an increase in magnitude with the peak response appearing during the middle of the experiment (fourth and fifth infestations) at which time the standard deviations increased. The peak coincided with corresponding depressions in the levels of packed cell volumes and red blood cell counts (and HB). The subsequent levels of MCV remained well above the original status indicative of a persistent stimulus for release of bigger cells. The mean values of the mean corpuscular haemoglobin concentrations (MCHC) showed minimal variation within normal range but also steadily declined and original levels were not recovered (Fig. 7.7).

In all erythrocytic indices the pre-infestation mean values differed and were higher than those attained in successive infestations and these original levels were not recovered. The response in all indices, save the mean corpuscular haemoglobin concentrations, indicated low regenerative activity which declined in the final stages of exposure. The tick numbers fed during successive infestations (Fig. 3.3) do not bear a consistent relationship with the haematological values. Thus during the fourth infestation tick recovery dropped markedly, at which time the indices also dropped, yet during fifth exposure both the fed ticks and indices increased. Then during subsequent infestations they appeared concurrently in both the tick numbers and the haematological indices. Nonetheless, the induced changes demonstrate the chronic effects caused by successive nymphal feeding.

FIG. 7.5

Means and standard deviations of Haemoglobin
Concentrations of five calves exposed weekly to successive
nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7.6

Means and standard deviations of Mean Corpuscular
Volumes of five calves exposed weekly to successive
nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7.5

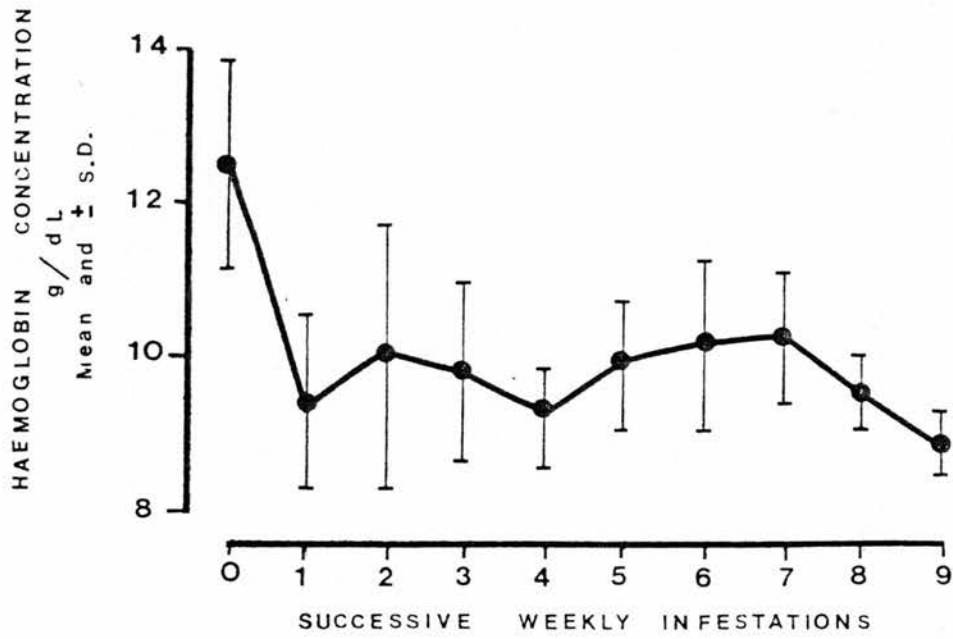


FIG. 7.6

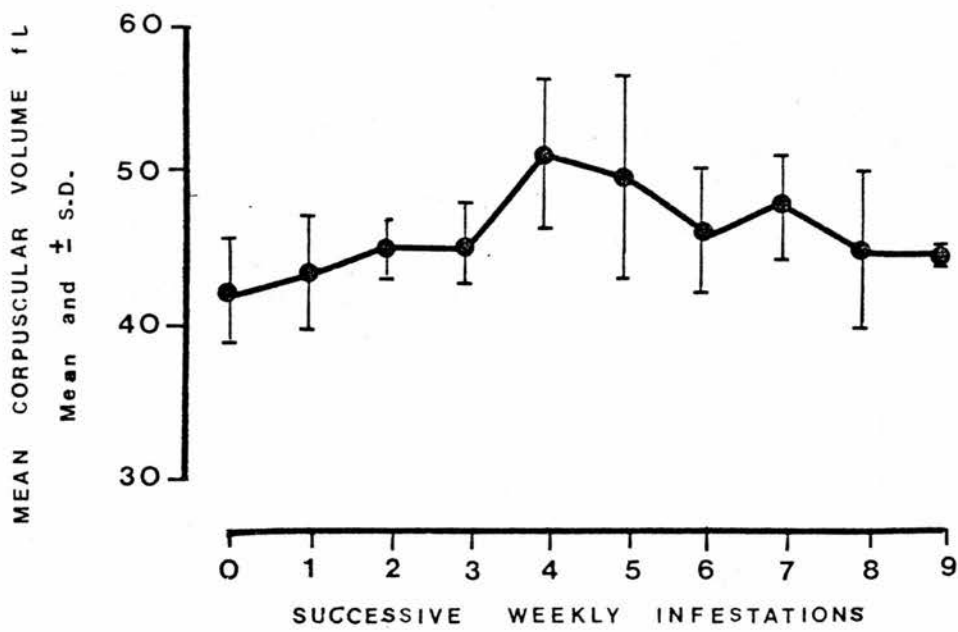


FIG. 7.7

Means and standard deviations of Mean Corpuscular
Haemoglobin Concentrations of five calves exposed
weekly to successive nymphal infestations of
Rhipicephalus appendiculatus.

FIG. 7.8

Means and standard deviations of White Blood Cell
counts of five calves exposed weekly to successive
nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7-7

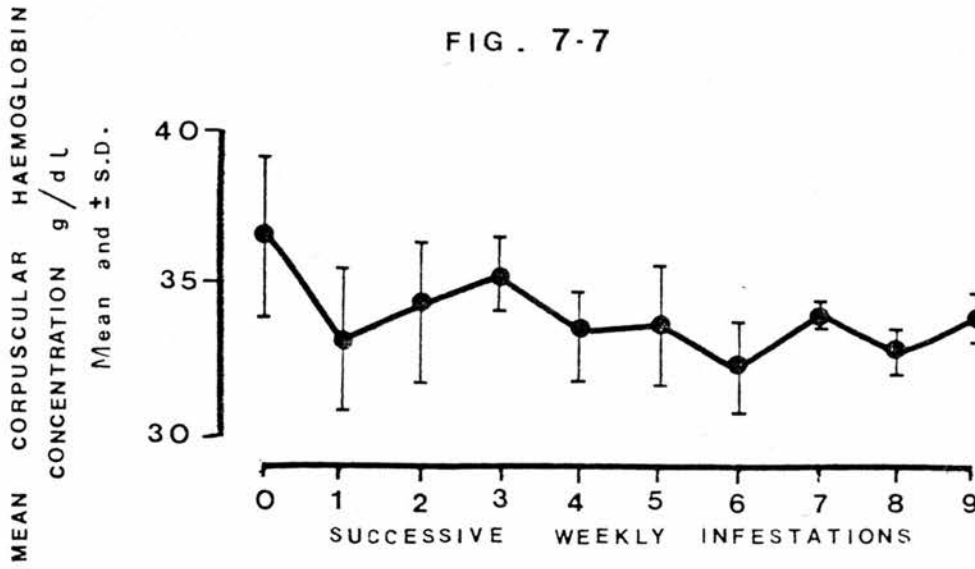
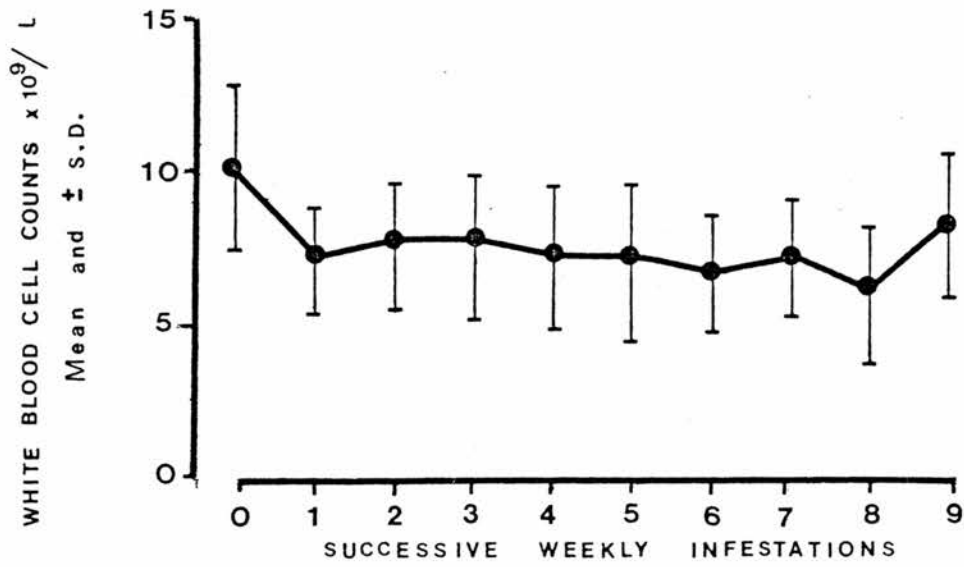


FIG. 7-8



Similarly the pre-infestation mean values of total white blood cell counts (WBC) (Fig. 7.8) and the absolute differential counts (Figs. 7.10 & 7.11) were higher than those obtained during successive nymphal infestations. However, the absolute counts of neutrophils (Fig. 7.9) and eosinophils (Figure 7.11) showed higher levels subsequently. Nonetheless, the mean values of all series were within normal ranges and their standard deviations small except a wider variation appeared in the neutrophil counts. The cause for these wide variations is not clear, for the clinical inspection does not reveal reactions attributable to bacterial infection. The variations appear to have maintained the uniformity in the WBC values whilst the lymphocyte count values assumed a relatively steeper decline (Fig. 7.10). Despite the anomaly the neutrophil response showed no significant difference between all experimental groups. The eosinophil cell count values were quite low and not characteristic of response to parasitism. As suggested before (C.5.2.) the levels of eosinophils could have been affected by sampling when the peak stimulus from nymphal feeding had passed.

7.2.2. The analysis of variance, as described before (C.4.3.2) revealed significant differences between the uninfested control group (I) and other experimental groups, including this group (IV), in all parameters estimated (Tables 4.1 to 4.9), except mean neutrophil counts (Table 4.7). However, the group was not included in analyses

FIG. 7.9

Means and standard deviations of absolute Neutrophil
Cell counts of five calves exposed weekly to successive
nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7.10

Means and standard deviations of absolute Lymphocyte
Cell counts of five calves exposed weekly to successive
nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7-9

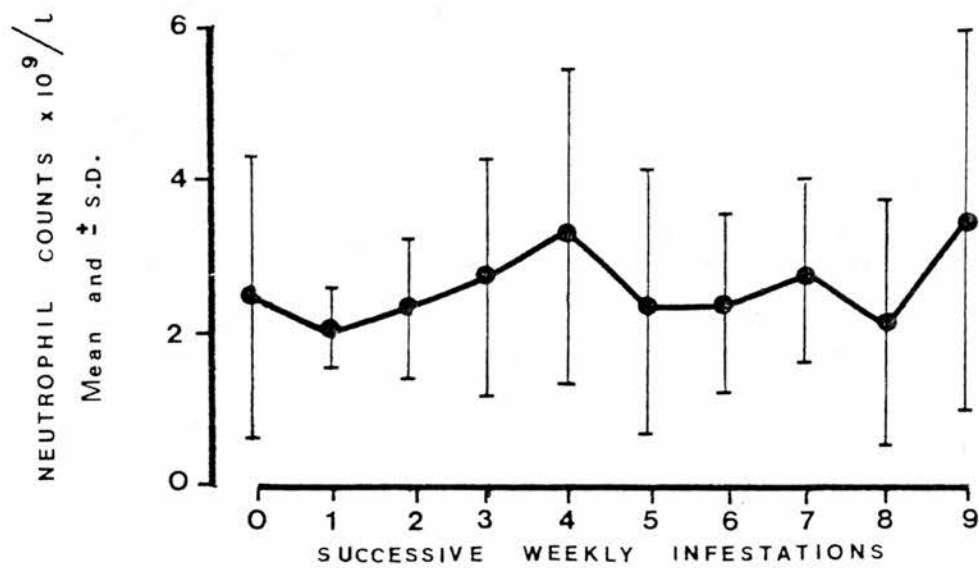
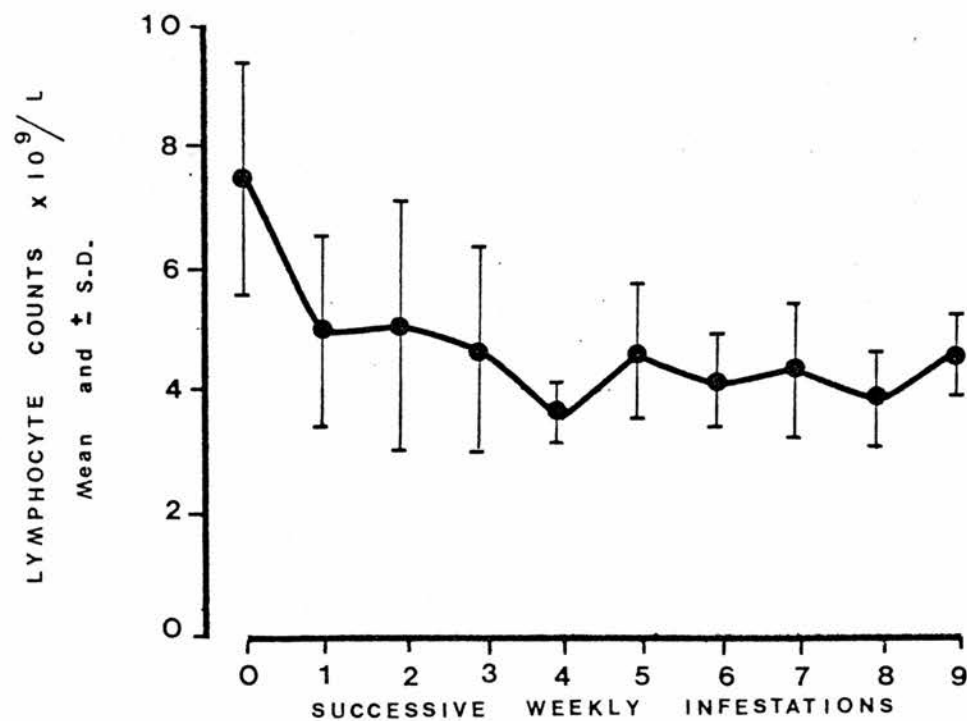


FIG. 7-10



for the tenth infestation, as no samples were taken for technical reasons, and in other analyses where no eosinophil cells were found (Table 4.9). The levels at which significance was revealed and their frequency are described under C.4.3.2.

The Duncan's New Multiple Range test (DNMRT) similarly eliminated the differences by resolving significant subsets illustrated in Tables 4.10 to 4.17. The group (IV) constituted similar response subsets and also overlapped between subsets. The test ranked the group in the similar subsets as follows. The lowest values ranking at greater than 50% of the analyses showing significant variance, was attained only in mean absolute lymphocyte counts (Table 4.16). Similarly lowest values ranking at less than 50% of the analyses was attained in the mean levels of each of packed cell volumes, haemoglobin concentrations, total white blood cell counts and absolute eosinophil counts (Tables 4.10, 4.12, 4.15 & 4.17). Conversely the highest values ranking at less than 50% of analyses was attained only in the mean levels of mean corpuscular volumes (Table 4.13). However, an intermediate position within the subsets with lowest or lower values at greater than 50% of the analyses showing significant variance was attained in each of red blood cell counts, total white blood cell counts and at less than 50% of the analyses was attained in each of packed cell volumes, haemoglobin concentrations (HB)

and absolute lymphocyte counts. Thus the test revealed that the frequency of closest association during periods of variance was highest with group (V) (infested with larvae) followed by groups (III), (II), (VI) (infested with concurrent three instars) and (I) (uninfested controls) in descending order. Group (VI) would have assumed a closer position but estimations were only for three successive weeks. It is noteworthy that the test aligned the group (IV) closest to group (V) indicating the immature instars at the densities applied provoked almost identical responses. Nonetheless, the chronic effects induced appreciable degeneration of the indices which drew the group toward group (III) (infested with high density adults). This is attributable to the much higher percentage recovery rates in the engorged nymphs (Fig. 3.5).

7.2.3. Biochemical estimations.

The mean values and standard deviations of serum levels of protein (S.P.), immunoglobulins (IG) and glucose (S.G.) are shown in Figures 7.12 to 7.14. The estimations for serum glucose were limited by supplies of reagents. Initially the serum protein gave low mean normal values that declined followed by gradual transient recovery. However, a sharp drop to suboptimal levels appeared in the least two weeks (Fig. 7.12). The standard deviation values were big. The response shows that the nymphal feeding exerted appreciable inhibitory effect on the serum protein

FIG. 7.11

Means and standard deviations of absolute Eosinophil
Cell counts of five calves exposed weekly to successive
nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7.12

Means and standard deviations of Serum Protein
levels of five calves exposed weekly to successive
nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7-11

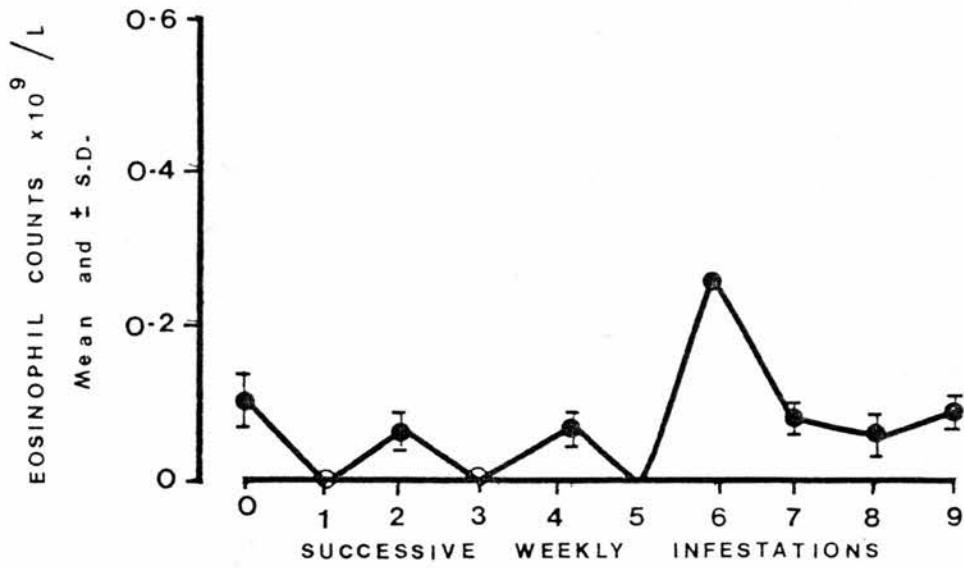
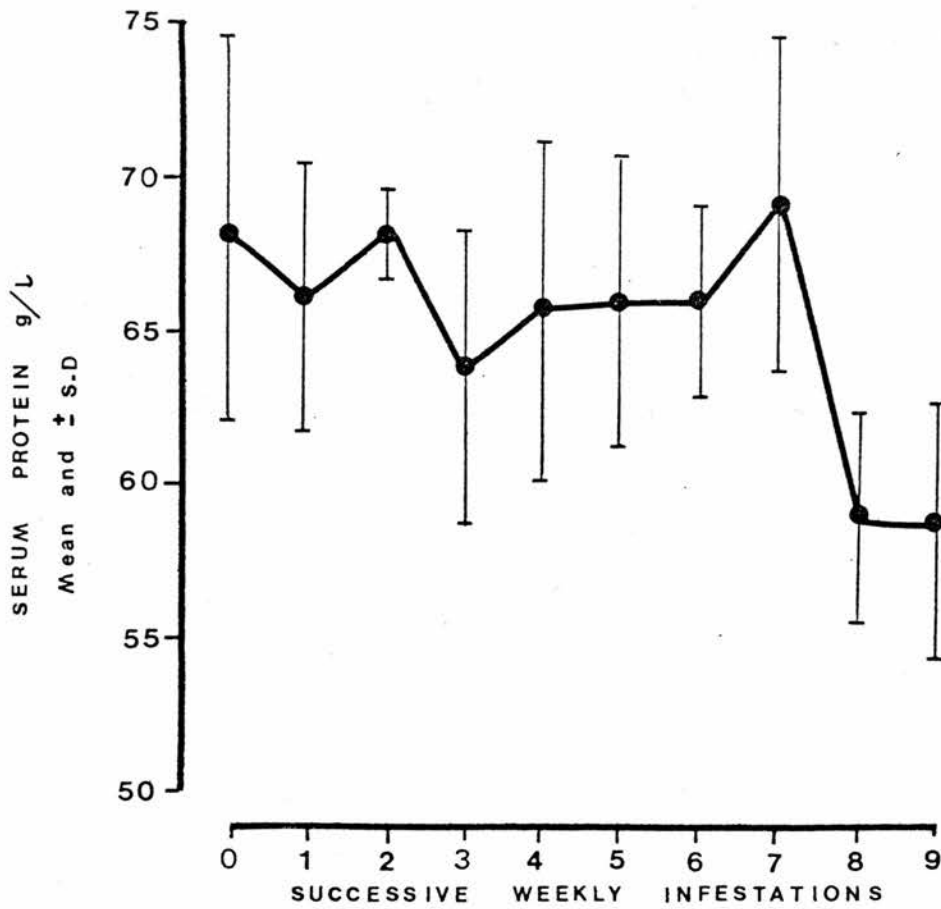


FIG. 7-12



levels despite calves feeding well. The mean values of immunoglobulins similarly presented low safe levels throughout and their standard deviations were generally small. However, the suboptimal mean value and wide standard deviation during first infestation could not be explained, for repeated estimations on the samples gave consistently extremely low concentrations for two calves. Thus the level of the mean was depressed by the readings from these calves. The low levels in both serum protein and immunoglobulins could mean reduced feed conversion, subtle decay through tick feeding effects or poor immunoglobulin production. The serum glucose concentrations were generally high and varied little between individual calves. The levels showed^a/tendency to rise and at the last estimation the mean values were higher than the original values (Fig. 7.14). The depression at third infestation coincided with a marked percentage recovery (80%) in engorged nymphs (Fig. 3.5) though the absolute number recovered was relatively low (Fig. 3.3). Thus the numbers of engorged nymphs recovered did not show significant correlation with serum protein estimations during successive infestations ($r = 0.32$, $p > 0.05$).

7.2.4. Analysis of variance revealed significant differences between the uninfested control group (I) and other experimental groups, including this group (IV), and the results are described in C.4.3.2 (Tables 4.18 to 4.20).

FIG. 7.13

Means and standard deviations of Immunoglobulin levels of five calves exposed weekly to successive nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7.14

Means and standard deviations of Serum Glucose levels of five calves exposed weekly to successive nymphal infestations of Rhipicephalus appendiculatus.

FIG. 7-13

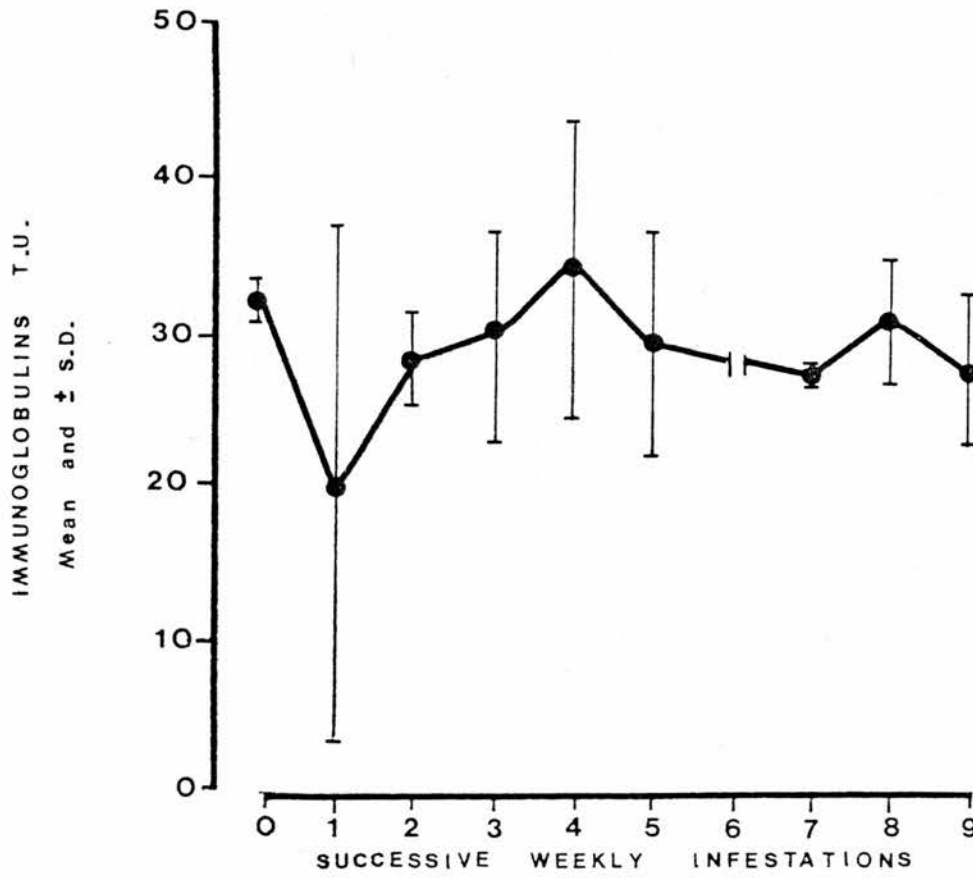
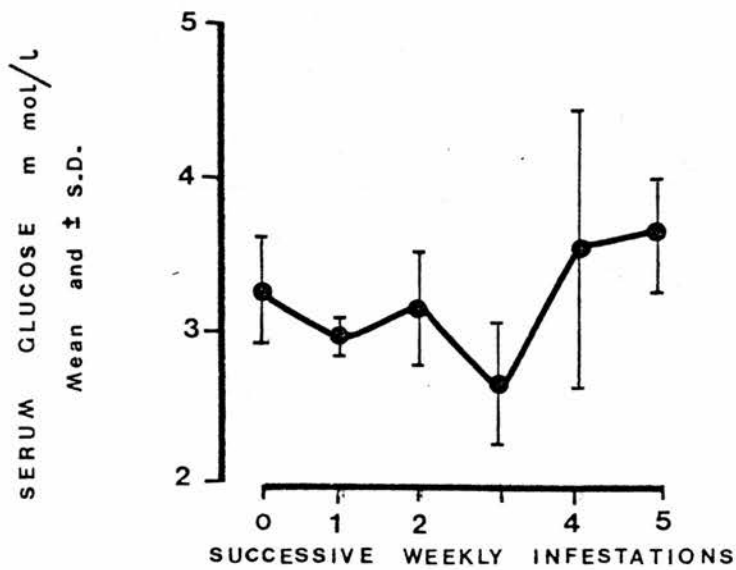


FIG. 7-14



Duncan's New Multiple Range test resolved significant subsets in which the group (IV) responded similarly with other groups and also overlapped between subsets (Tables 4.21 to 4.23). The group attained highest value ranking in one subset (Table 4.21) during second infestation, being identical to ^{the} position before infestation. This shows the serum protein levels had not been seriously affected then. On the other hand, the group assumed different ranks within the overlapped subsets, thus it appeared in both high and low rank subsets. The test ^{that} showed/the frequency of very close association was with group (III) followed by groups (II) (low density adult infestations) and (V) (larval infestations), but appeared furthest from group (I) (uninfested controls). Overall the nymphal feeding induced responses which differed from those in the uninfested control group revealed by the above ranking.

7.3. AUTOPSIES

No natural deaths occurred, but autopsies were performed on calves slaughtered at the end of the experiment. The gross picture of the internal organs did not reveal significant changes except in regional drainage lymph nodes i.e. parotids, and poor fat distribution. Changes were slight enlargement, oedema and occasional patchy congestion of the parenchyma. These changes were attributed to direct effects caused by tick feeding. However,

the skin in the infested areas, especially ears, still showed scars, alopecia and extensive pityriasis.

7.4. SUMMARY OF SIGNIFICANT FINDINGS

The densities of nymphs applied induced tangible changes in the health of the calves. There was moderate loss in body condition, but the calves remained bright and active. Pain and irritation, especially during secondary infestations, caused behavioural and nervous reactions, e.g. restlessness, grooming, reflex urination. The cardiovascular changes were manifested systemically and in the skin and visible mucous membranes. Systemic response revealed mild transitory pyrexia, the skin bore erythema, oedema and exudation; the mucosae produced excessive secretions and were congested. Lymphadenopathy developed in regional lymph nodes draining infested areas. The ears sustained extensive dermatitis accompanied by exudation/encrustation which culminated in alopecia and scarring. Rupture of blebs considerably augmented the exudation from bite wounds. The skin of the ears developed characteristic exudative bleb lesions of papule/pustule type indicative of hypersensitization to salivary secretion of Rhipicephalus appendiculatus nymphs. The mean haematological and biochemical readings were slightly affected and remained within normal ranges. However, significant variance was revealed

between the uninfested control group and other experimental groups, including this group (IV) (infested with nymphs) in the clinical-pathological estimations. Further analysis associated the group most closely with groups (V) (infested with larvae) and (III) (infested with high density adults) in the clinical-pathological responses. Due to overall moderate effects on the health of the animals no deaths occurred but autopsy on slaughter revealed lack of carcass fat and mild inflammatory changes in the regional drainage lymph nodes.

CHAPTER 8

EXPERIMENT (V): RESPONSES OF FIVE CALVES EXPOSED WEEKLY
TO SUCCESSIVE INFESTATIONS OF LARVAL RHIPICEPHALUS
APPENDICULATUS

The calves aged three to six months and with no previous experience of tick effects were clinically healthy at the first infestation of larvae of Rhipicephalus appendiculatus. The mean numbers of the applied (unfed) and recovered engorged larvae and standard deviations of the latter are presented in Fig. 3.4. The densities of larvae applied provoked appreciable reactions, especially in the cardiovascular, nervous and integumentary systems. But no abnormalities were detected in urinary function which agreed with observation on reactions to nymphal exposure. However, the responses were generally mild and caused no serious decline in the clinical condition of the calves. The clinical observations and clinical-pathological estimations made during the successive infestations revealed the following manifestations.

8.1. PHYSICAL (CLINICAL) SIGNS

8.1.1. Attachment of ticks caused marked immediate reactions in calves during other infestation regimes, but the attachment of the larvae provoked no significant response and all animals withstood the infestation until much later on the first day. Later on

during the first day the development of swelling and pain of the pinna as the larvae continued feeding, caused frantic head/ear shaking and marked effort to groom. This appeared predominantly in the secondary infestations and when the applied density exceeded an estimated 40 000 larvae per infestation. The other clinical features accompanying established attachment and feeding were associated with the following manifestations.

8.1.2. Slight loss in body condition assessed by muscle wasting occurred in four out of the five calves especially during the last two infestations. On the whole the calves withstood the infestations reasonably well and for most time appeared bright and active. However, their demeanour and physique were affected in the last infestations when they developed some degree of lethargy.

8.1.3. Cardiovascular system showed mild to moderate reactions in the various parameters. A mild and transient pyrexia was induced. There was no consistent elevation in the temperature response of the individual calves, for raised levels were recorded on different days. Thus the mean weekly rectal temperatures illustrated in Fig. 8.1 were below the critical normal level (39.3°C) except in the last infestation when slight pyrexia occurred. The compared mean temperatures of the uninfested controls showed slight pyrexia initially but this was due to non-specific factors. The distinction between the compared

FIG. 8.1

Mean weekly Rectal Temperatures of five calves exposed weekly to successive larval infestations of Rhipicephalus appendiculatus compared with uninfested controls. Arrow marks normal critical temperature.

FIG. 8.2

Mean daily Rectal Temperatures of five calves exposed to tenth successive weekly larval infestation of Rhipicephalus appendiculatus compared with uninfested controls. Arrow marks normal critical temperature.

FIG. 8·1

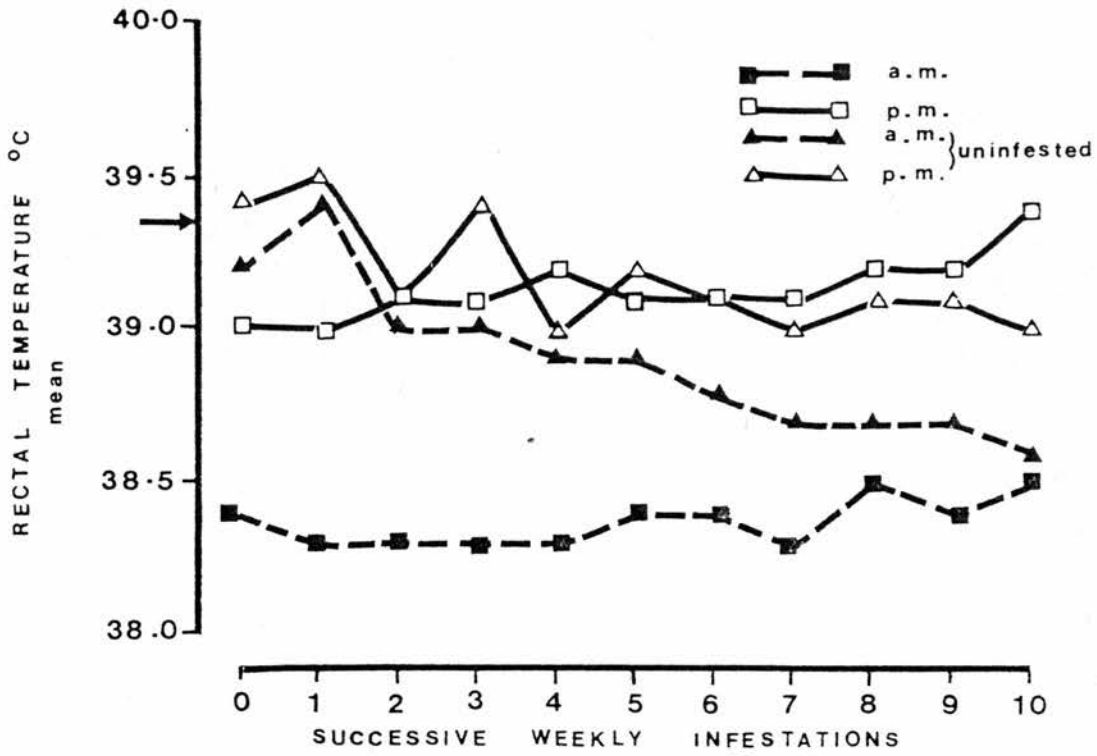
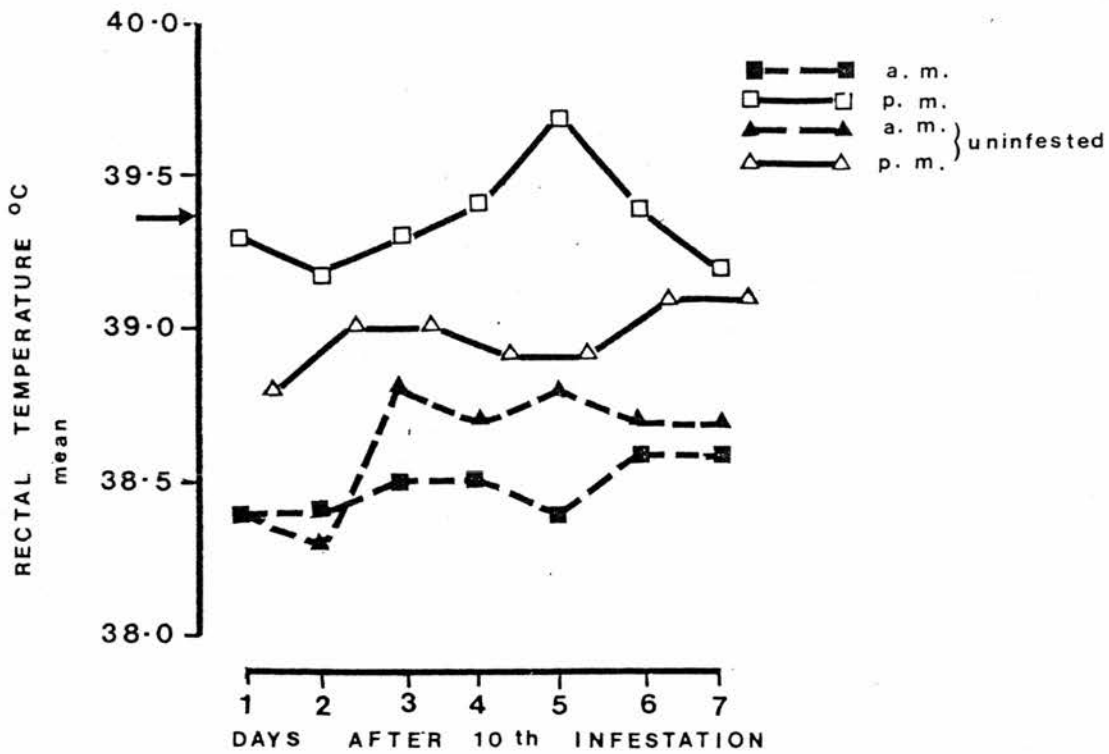


FIG. 8·2



groups is demonstrated by mean readings of p.m. temperature, after fourth exposure, which steadily rose in the larval infestations whereas in the controls there was greater fluctuation accompanied by lower levels ultimately. The pyrexia peak at tenth infestation in the successive mean weekly temperatures is amplified in Fig. 8.2. The amplification shows the mean daily temperatures during the week. The slight pyrexia peak revealed coincided with engorgement of larvae (three to five day post infestation) though the recovered engorged larvae were lower than in some of the previous recoveries (Fig. 3.4.) when no pyrexia appeared. Thus of the individual instar infestations of R. appendiculatus, the larval regime provoked the least mean pyrexia reaction. However, it is noteworthy that a genuine reaction occurred in the last infestation in contradistinction with other regimes which produced marked reactions initially and regressed to base subsequently. Mild to moderate rises appeared in the heart and pulse rates during one to three infestations in individual calves. The reaction occurred in four calves which sustained severe oedema, bleb formation and pruritis. This indicates/that the pain and irritation were major stimuli. The mucous membranes showed occasional congestion of conjunctivae and infrequent excessive ocular, nasal and salivary secretions. The ocular and nasal secretions became mucopurulent at times. The conjunctival congestion occurred predominantly on the side infested. Inspection of the mouth cavity, pharynx and related parts of the head revealed no visible lesions to associate with

the salivation. This profuse salivation from no apparent clinical reaction was thus observed in four infestation regimes (C.5.1., C.6.1.5, C.7.1.5 & C.8.1.3). Erythema developed over infested ears preceding the oedema and bleb formation. The oedema was confined to the ears and was most severe in two out of five calves associated with hypersensitivity bleb formation. Thus the marked responses occurred during the secondary infestations and most cases subsided shortly after complete repletion of the larvae.

8.1.4. Lymphadenopathy was not a prominent feature as compared with other infestation regimes. No significant enlargement could be measured during the initial infestations but three out of five calves showed mild reaction in the second half of the experiment. In two of the calves the enlargement was estimated at two to three times the original size at each infestation. The reaction subsided shortly after infestation. Further inspection of the results revealed that these calves yielded greater recoveries overall during the successive infestations. Thus the enlargement of the lymph node was associated with the high percentage of recovered nymphs during later exposures (Fig. 3.5). Reappraisal of response to infestation regimes of individual instars of R. appendiculatus shows a gradation of the lymphadenopathy whereby the response was most severe with adults and least with larvae whilst the nymphs assumed the intermediate position.

8.1.5. The digestive system functioned normally throughout the successive infestations, but one calf developed mild diarrhoea in two infestations. The diarrhoea developed concurrently with elevated rectal temperature, severe bleb formation, oedema and pain; nonetheless the appetite was not affected. The diarrhoea could have developed through nervous irritation associated with other manifested symptoms. In all calves, the nervous system showed varied degrees of pruritis which was observed on three to seven occasions in individuals during the infestations. On a few occasions pain and irritation led to head/ear shaking, stamping and restlessness while calves remained restrained. However, restraint minimised the abrasions caused by grooming. In review, pruritis was common to all infestation regimes of individual R. appendiculatus instars and the intensity was almost the same allowing for influence of the restraint.

8.1.6. The integumentary system relative to other infestation regimes was affected markedly. Bite wounds, as the primary lesion, varied in severity between and on individuals during the successive exposures but were generally mild. Massive clustering of feeding larvae induced excessive exudation that formed thick crusts and scabbing. Mild loss of hair occurred in badly wounded or encrusted areas, and delayed drying of the exudate led to 'scalding' of the skin and further loss of hair. Repeated infestation over individual ears provoked protracted pityriasis that made the ear surface scurfy.

Bleb formation occurred in all calves and, in contrast with the response to nymphs, the lesions were predominantly of the pseudovesicle type. Coalescing lesions produced big bullae over a large portion on both sides of the ears. The blebs were most severe in two calves and overall appeared as early as three to six hours after application of larvae in individual calves. Rupture of the blebs released copious plasma, which formed fibrin clots inside the sleeves that virtually killed all attached larvae, even the unattached ones were unable to escape from the confines of the sleeves. Cytology on the fluid from the blebs showed scattered healthy neutrophils and few lymphocytes. The low concentration and types of leucocytes was possibly limited by cells being held in the surrounding tissues and not lying free in the fluid. This suggestion is supported by the extensive histological studies on skin from tick-infested animals which have revealed dense infiltration of epidermis/dermis with basophils, eosinophils etc. (Schleger et al., 1976, Tatchell & Moorhouse, 1968). Another possible explanation given by Schleger et al., (1976) is the poor staining of the degranulated cells which makes them difficult to find. The Giemsa stain, I used, does not always reveal distinct features in such cells. The bleb formation appeared only during secondary infestations and was therefore characteristic of a hypersensitivity state. Healing of uncomplicated lesions was rapid under the encrustation/scabs. It is noteworthy that the detachment wounds in severely infested ears continued to release exudate which formed secondary encrusts superimposed on the preceding layers.

The relatively mild clinical reactions to such high numbers of larvae could be ascribed to the minute size of individual ticks and the short feeding period, as both restrict the blood meal. Nonetheless, dense clustering provoked serious dermatitis and concurrently induced a marked cutaneous hypersensitivity reaction associated with bleb formation.

8.2. CLINICAL-PATHOLOGICAL FINDINGS

8.2.1. Haematological estimations.

The mean weekly absolute values and standard deviations of the estimated parameters are presented in Figs. 8.3 to 8.11. The mean levels of erythrocytic indices were within normal ranges (Figs. 8.3 to 8.7) and the levels during successive infestations were lower than the pre-infestation estimations, except for the mean corpuscular haemoglobin concentrations (MCHC) and mean corpuscular volumes (MCV) (Figs. 8.6 & 8.7). The packed cell volumes, red blood cell counts and haemoglobin concentrations values showed similar fluctuation. Their lowest mean values and corresponding rise for the mean corpuscular volumes coincided with the highest mean larval infestation (Fig. 3.4.). Nevertheless, the mean recovery of engorged larvae was very low. The elevated mean corpuscular haemoglobin concentrations and corresponding big standard deviation during the sixth estimation

FIG. 8.3

Means and standard deviations of Packed Cell
Volumes of five calves exposed weekly to successive
larval infestations of Rhipicephalus appendiculatus.

FIG. 8.4

Means and standard deviations of Red Blood Cell
counts of five calves exposed weekly to successive
larval infestations of Rhipicephalus appendiculatus.

FIG. 8-3

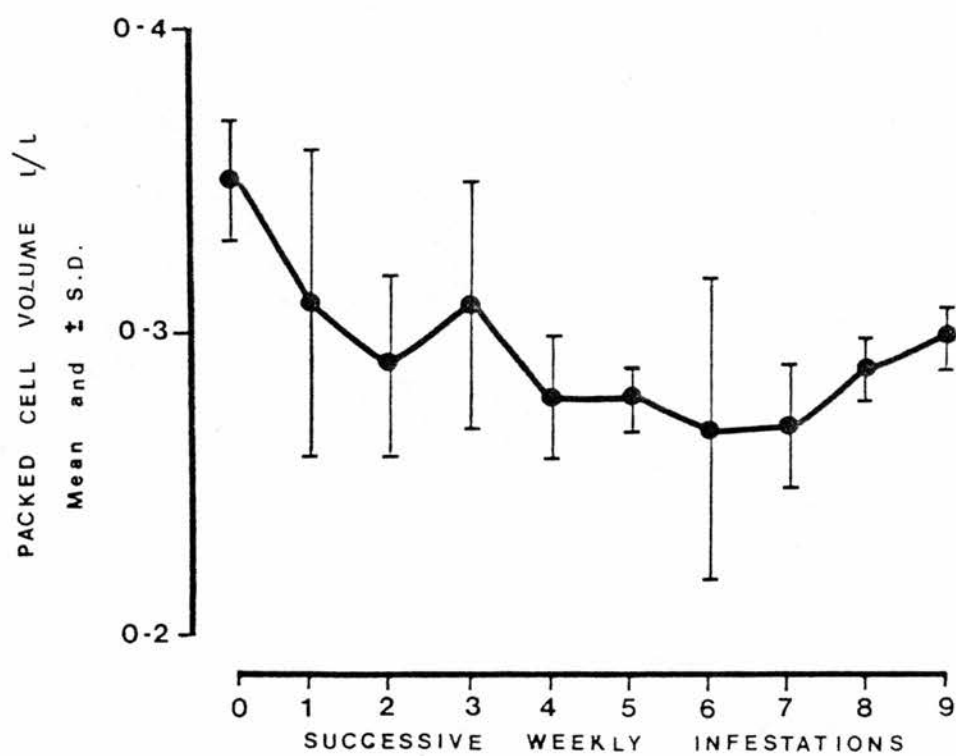
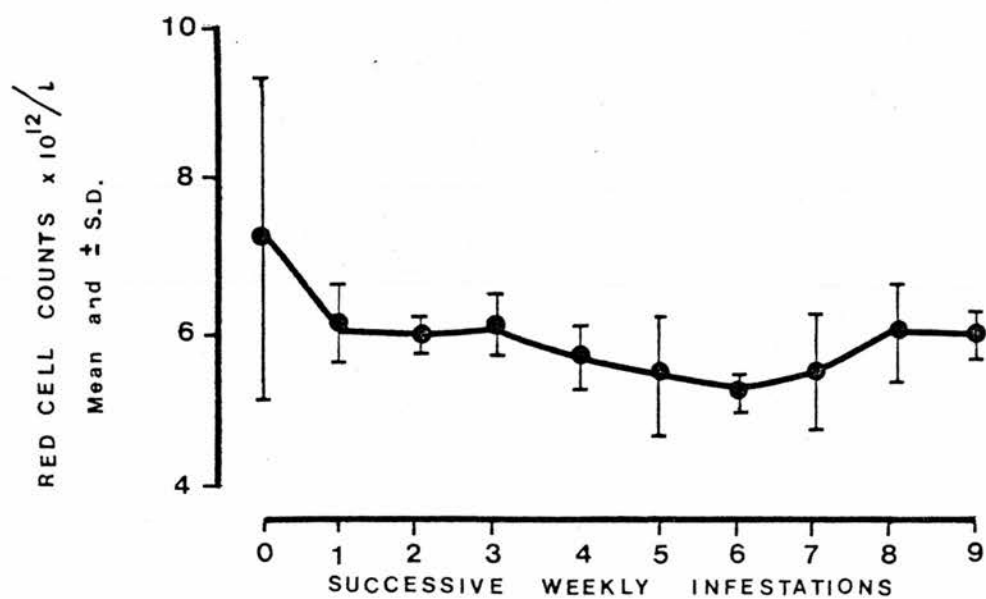


FIG. 8-4



was caused by technical difficulty. The drop in the normal values can be attributed to chronic effects of infestation. But the inhibited response cannot be entirely associated with sucked blood for the faecal output and engorged weights obtained during the short feeding periods are relatively very low compared with the nymphal effects. However, the statistical analyses revealed both groups (IV) (nymphal infestations) and (V) (larval infestations) were closely associated in their haematological responses. It can be suggested that the effects of the by-products of larval feeding played a major role but the mechanism is not clear.

In the leucocytic series (Figs. 8.8 to 8.11) the mean absolute values were within normal ranges. The levels for the first infestation samples were higher than the pre-infestation ones but declined later. However, the mean levels of total white blood cell counts (WBC) (Fig. 8.8) and absolute neutrophil counts (Fig. 8.9) showed a contrasting sharp rise during the eighth infestation which was associated with similar increase in the mean recovery rate of engorged larvae (Fig. 3.4). The eosinophils were infrequent and very low in absolute numbers. As stated before the levels were back to normal since samples were taken some days after fed ^{the} larvae had detached. In/absence of the feeding ticks the stimulus for eosinophilia mediated through sensitization reactions will be lacking. Overall fluctuations

FIG. 8.5

Means and standard deviations of Haemoglobin
Concentrations of five calves exposed weekly to successive
larval infestations of Rhipicephalus appendiculatus.

FIG. 8.6

Means and standard deviations of Mean Corpuscular
Volumes of five calves exposed weekly to successive
larval infestations of Rhipicephalus appendiculatus.

FIG. 8-5

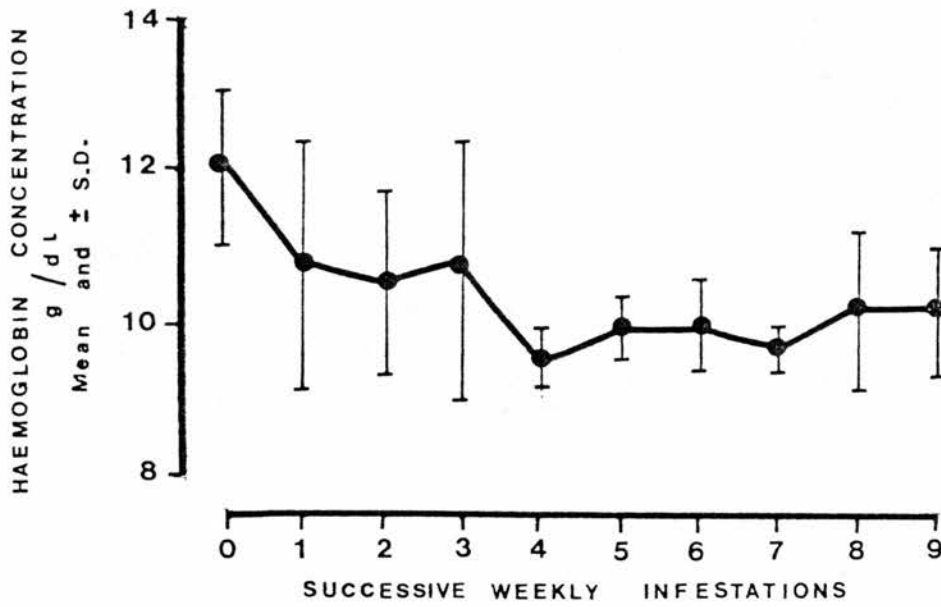


FIG. 8-6

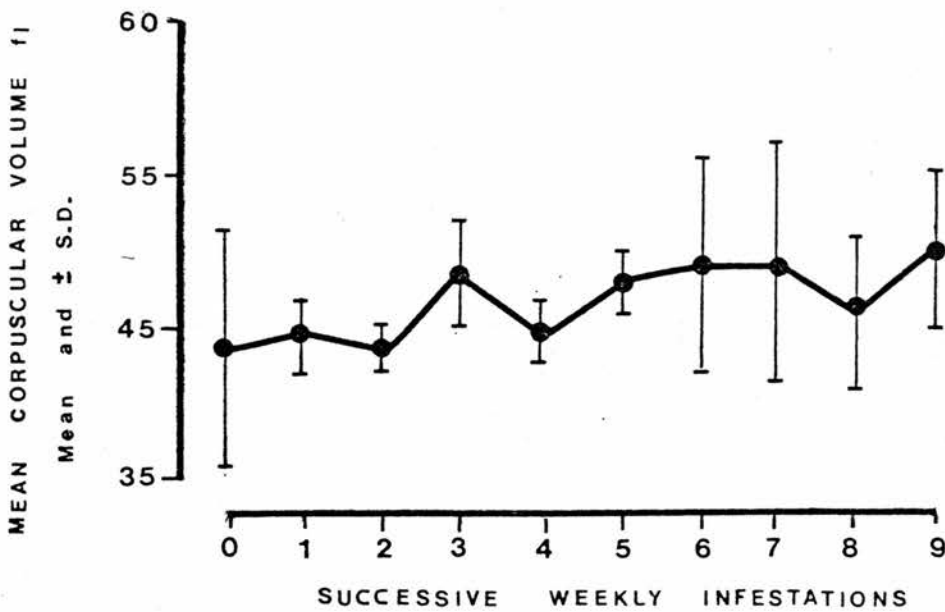


FIG. 8.7

Means and standard deviations of Mean Corpuscular
Haemoglobin Concentrations of five calves exposed
weekly to successive larval infestations of
Rhipicephalus appendiculatus.

FIG. 8.8

Means and standard deviations of White Blood Cell
counts of five calves exposed weekly to successive
larval infestations of Rhipicephalus appendiculatus.

FIG. 8-7

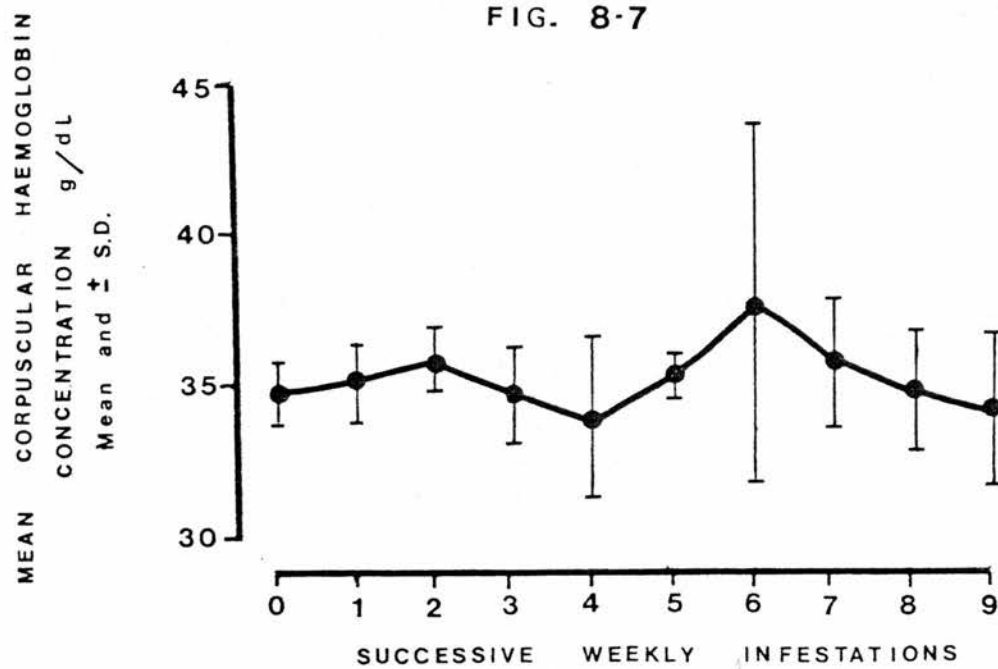
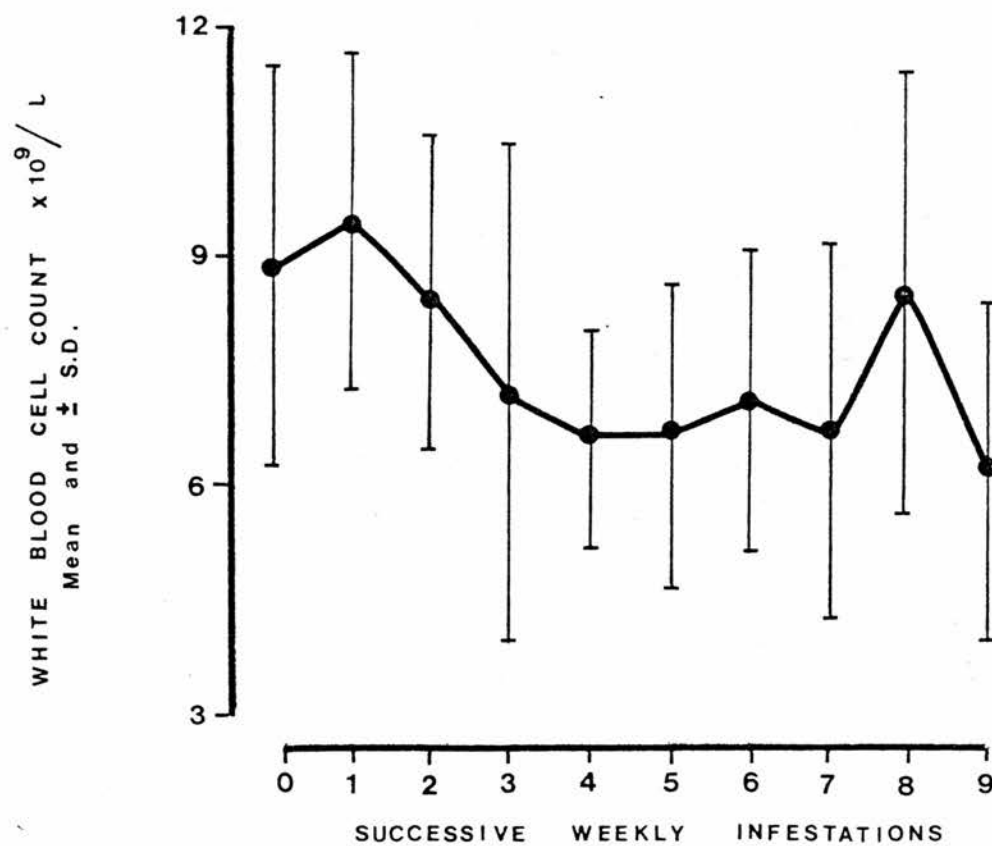


FIG. 8-8



were small except for total white blood cell counts (WBC) which showed big standard deviations. The marked standard deviations in WBC matched by absolute neutrophil counts cannot be explained as no specific reactions capable of inducing such responses were detected in individual calves.

8.2.2. Analysis of variance revealed significant differences between the uninfested control group (I) and other experimental groups including this group (V), in all estimated parameters (Tables 4.1 to 4.9) except mean neutrophil counts (Table 4.7). However, the group was not included in all analyses for the tenth successive infestation as no samples were taken and in another analysis where eosinophils were not found (Table 4.9). The frequency and levels of variance are described in C.4.3.2.

Duncan's New Multiple Range test (DNMRT) eliminated the revealed variance by resolving significant subsets illustrated in Tables 4.10 to 4.17. The test ranked the group in subsets where different groups responded similarly and also overlapped between subsets for particular parameters in the successive estimations. The test revealed the group (V) attained the highest mean values once in the mean corpuscular volumes (MCV) during third infestation, (Table 4.13) but that coincided with the lowest tick load. Conversely the lowest mean values in the ranking appeared once in each of white blood cell counts (WBC), absolute differential counts of lymphocytes and

FIG. 8.9

Means and standard deviations of absolute Neutrophil
Cell counts of five calves exposed weekly to successive
larval infestations of Rhipicephalus appendiculatus.

FIG. 8.10

Means and standard deviations of absolute Lymphocyte
Cell counts of five calves exposed weekly to successive
larval infestations of Rhipicephalus appendiculatus.

FIG. 8.9

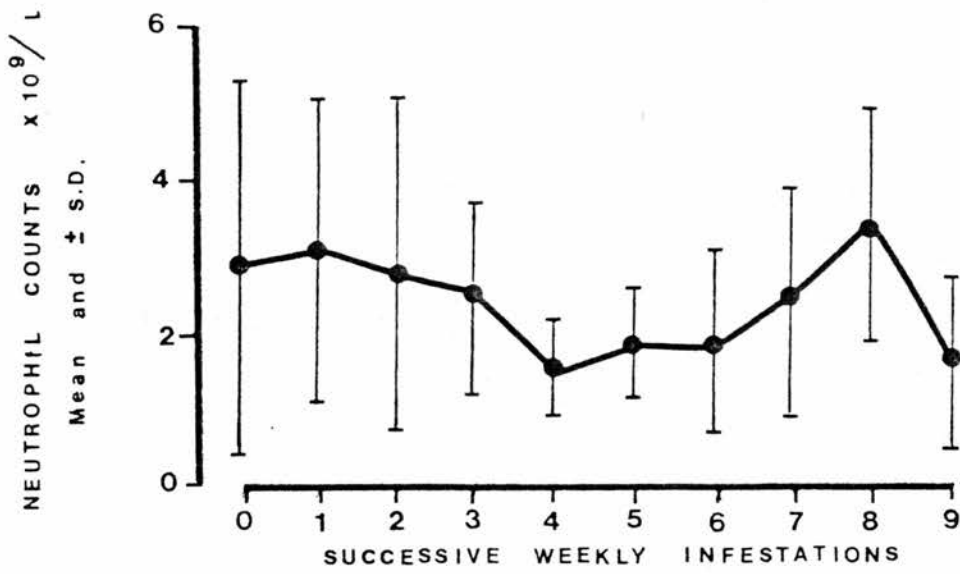
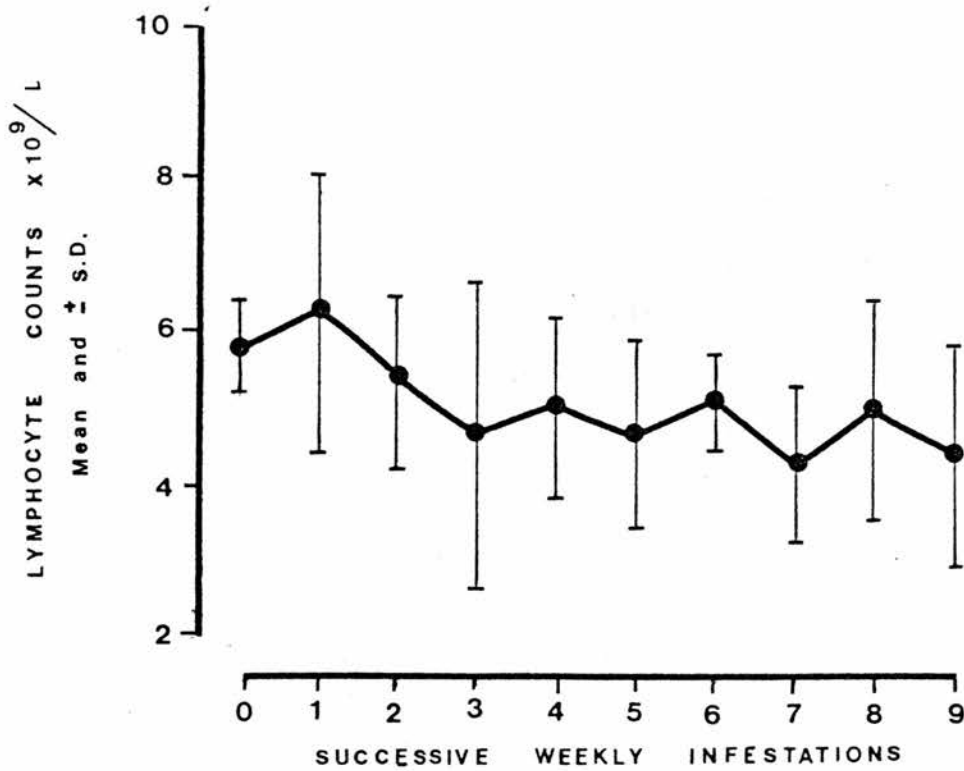


FIG. 8.10



eosinophils (Table 4.15 to 4.17) and these coincided with the highest means of estimated density of larvae applied (Fig. 3.4). The closest association with other groups showed highest frequency with group (IV) (infested with nymphs) followed by groups (III) (infested with high density adults), (II) (infested with low density adults) and (I) (uninfested controls) in descending order. The trend confirms the association shown in experiment (IV) (C.7.2.2). The less frequent association with the controls shows ^{that} the larvae altered the health status of the infested calves. The uninfested controls ranked predominantly in the higher values brackets. The next match of the adult infestation groups portrays the chronic effects though at one given infestation the response induced was of much lower severity.

8.2.3. Biochemical estimations

The mean values and standard deviations of serum concentrations of protein (S.P.), immunoglobulin (IG) and glucose (S.G.) from the weekly estimations are given in Figs. 8.12 to 8.14. All mean levels remained within normal ranges. The fluctuation demonstrated by the big standard deviations (Fig. 8.12) produced steady increases in the mean values of serum protein which were higher than the pre-infestation levels except for the first infestation when lower values obtained. This could be attributed to feeding/growth and low recoveries of engorged larvae. In contradistinction the mean values

FIG. 8.11

Means and standard deviations of absolute Eosinophil Cell counts for five calves exposed weekly to successive larval infestations of Rhipicephalus appendiculatus.

FIG. 8.12

Means and standard deviations of Serum Protein levels of five calves exposed weekly to successive larval infestations of Rhipicephalus appendiculatus.

FIG. 8-11

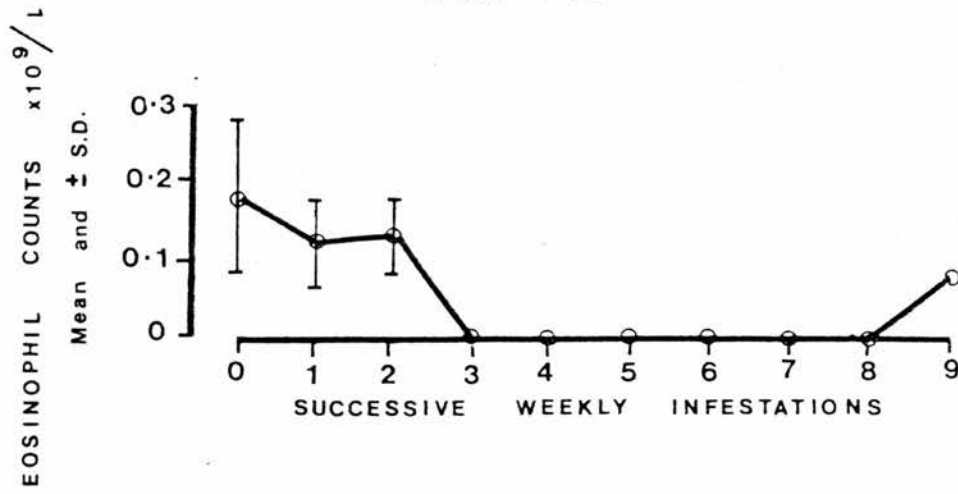
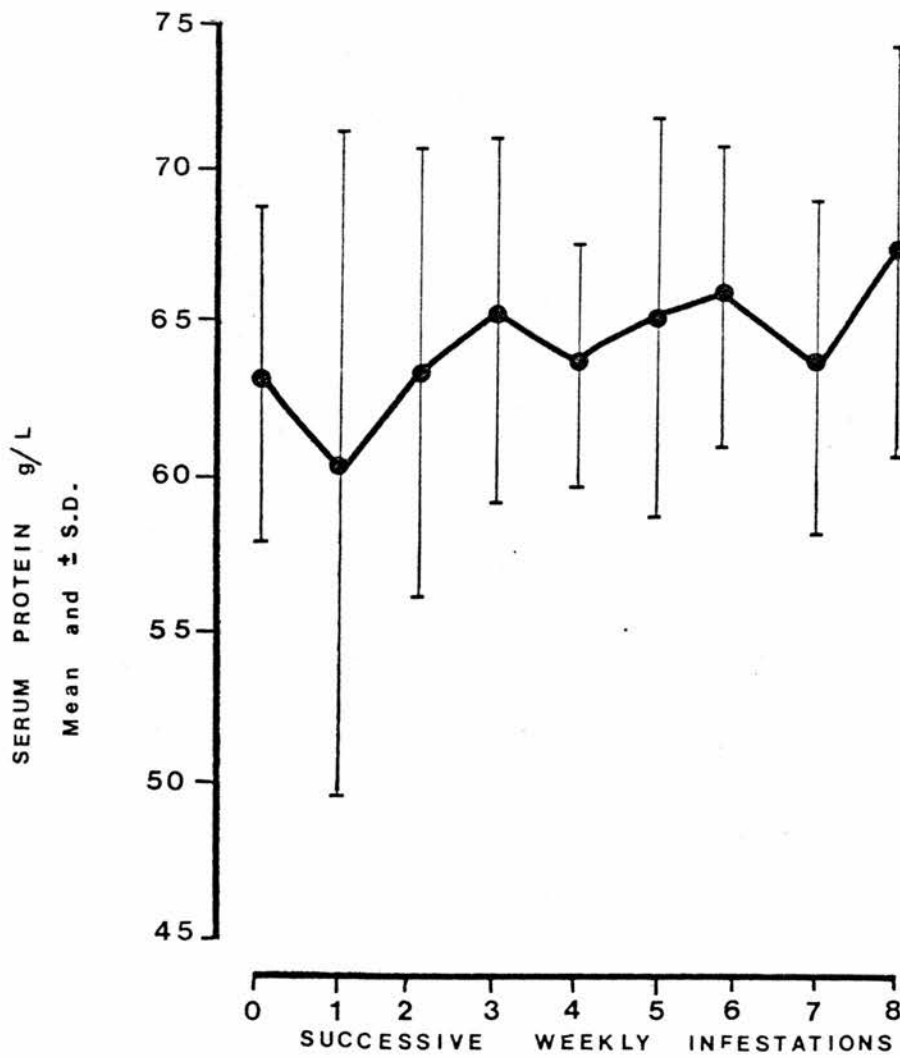


FIG. 8-12



of immunoglobulins declined steeply and never recovered the pre-infestation levels (Fig. 8.13). Their standard deviations were similarly much smaller. The initial decline in the mean values of the immunoglobulin could be associated with the waning of colostrum-derived globulins, whereas the subsequent rise in the values could be attributed to a response produced by the larval infestations. Nonetheless, the mean values of the immunoglobulin during infestations did not significantly differ between the infested and uninfested controls (Table 4.19). The mean values of serum glucose showed a similar trend to the serum protein values but the response in the former (Fig. 8.14) could be associated with the stress caused by repeated larval feeds. There was little variation in levels of individual calves as revealed by the small standard deviations.

8.2.4. Analysis of variance revealed significant differences between the uninfested control group (I) and other experimental groups, including this group (V) in the three indices estimated (Tables 4.18 to 4.20). However, the frequency of variance detected was low, being eight out of thirty three estimations, and three out of the eight occasions the differences appeared during the pre-infestation period. The differences revealed during exposure were confined to the first four infestations, however, in the eighth and ninth estimations for serum protein and glucose respectively significance just failed because of small degrees of freedom (Table 4.18, 4.20).

FIG. 8.13

Means and standard deviations of Immunoglobulin levels of five calves exposed weekly to successive larval infestations of Rhipicephalus appendiculatus.

FIG. 8.14

Means and standard deviations of Serum Glucose of five calves exposed weekly to successive larval infestations of Rhipicephalus appendiculatus.

FIG. 8-13

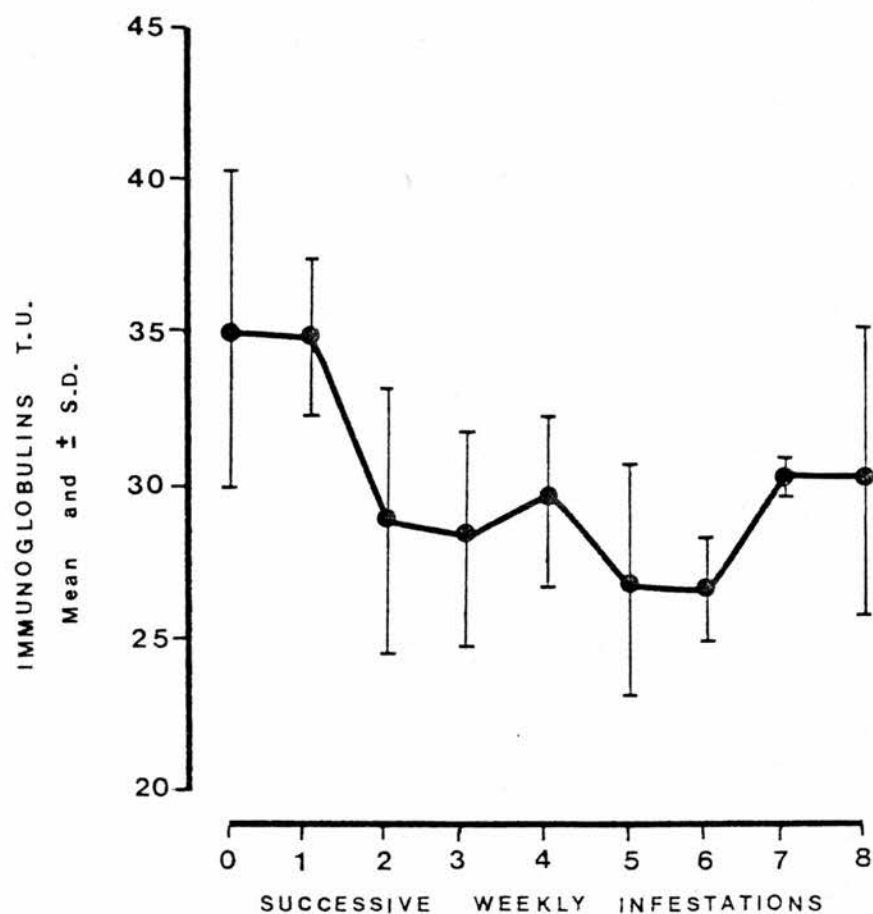
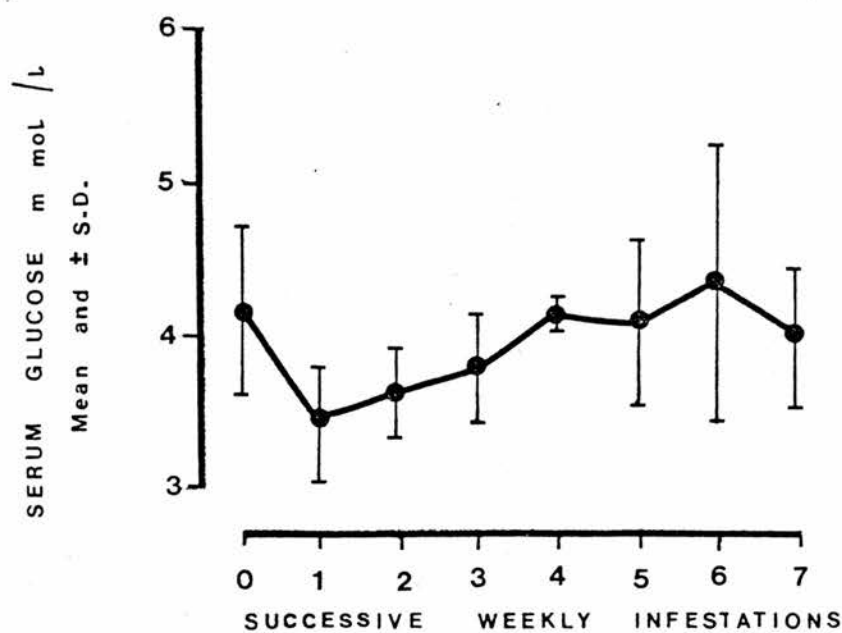


FIG. 8-14



Duncan's New Multiple Range test eliminated the differences revealed between groups in the few analyses by resolving significant subsets shown in Tables 4.21 to 4.23. The group constituted an independent and similar response subsets and also overlapped between subsets. The group (V) attained the highest value ranking on two occasions both in pre-infestation estimations (Table 4.22, 4.23). On the other occasions the group assumed an intermediate ranking within or between (overlap) subsets in which it did not assume the least rank in the lower (value) subsets. Thus the test revealed that the group suffered less than the other groups, such as, groups (III) and (VI) infested with high density adults and concurrent three instars of R. appendiculatus respectively. These groups showed the lowest values ranking during infestations. Overall the infrequent variance revealed between the control and other experimental groups in the three indices shows the infestation regimes used caused no drastic alteration in the mean responses of the calves. Therefore the indices were quite stable in withstanding the effects of tick feeding.

8.3. AUTOPSIES

As could be predicted from the responses to the successive infestations, the densities of R. appendiculatus larvae applied did not cause natural deaths.

The autopsies for comparison with other regimes were performed on four out of the five calves slaughtered twelve days after completion of the experiment. No gross pathological changes were detected except mild reduction in carcass fat, slight enlargement and oedema of parotid lymph nodes and marked pityriasis over ^{the} ears. Thus the levels of infestations had only slight effects on the gross appearance of carcass tissues compared with other infestation regimes. However, the response in lymph nodes and the skin (ears) were still distinguishable.

8.4. SUMMARY OF SIGNIFICANT FINDINGS

The successive infestations of Rhipicephalus appendiculatus larvae induced mild to moderate clinical-pathological responses in the calves. The most outstanding manifestations developed in the integumentary, cardiovascular and nervous systems. The cardiovascular system showed mild and transient pyrexia that coincided with engorgement of larvae during the final exposure. The visible mucous membranes showed occasional conjunctival congestion and increased secretions during different infestations. Erythema, oedema and exudation were induced in infested ears, especially during secondary infestations. Slight to moderate elevation in heart and pulse rates were provoked during severe reactions in the ears. The severe reactions included hypersensitivity blebs of pseudovesicle type which were accompanied by intense pain and pruritis and release of abundant

exudate. The blebs developed within six hours of infestation. Sequelae to the integumentary reaction caused by larval feeding were alopecia and pityriasis. Mild enlargement developed in lymph nodes draining the infested sites during the second half of exposure. Corresponding with the generally mild clinical manifestations, the mean haematological and biochemical levels showed minimal alteration of the normal ranges. However, significant differences and similarities between the uninfested control group and other experimental groups, including this one infested with larvae, were revealed in the clinical-pathological estimations. The variance between groups were infrequent in the estimated serum levels of protein, immunoglobulins and glucose. The calves withstood the larval infestations well and thus no death occurred. Autopsy on slaughter revealed no significant gross pathological changes except the distinguishable lesions of the ears and parotid lymph nodes.

CHAPTER 9

EXPERIMENT (VI): RESPONSES OF THREE CALVES EXPOSED WEEKLY TO SUCCESSIVE CONCURRENT THREE INSTAR INFESTATIONS OF RHIPICEPHALUS APPENDICULATUS.

Bos taurus calves aged about two months and not previously exposed to ticks were clinically healthy at the first concurrent three instar infestation of Rhipicephalus appendiculatus. The ticks were applied to ears and other parts of the body, facilitated by deep narcosis. Narcosis served to arrest grooming during attachment as ticks were applied to sites which could not be protected with calico sleeves. However, the calves were restrained subsequent to recovery from narcosis. Application of the ticks to other sites besides the ears partly aimed at preventing heavy clustering over relatively small area which would interfere with proper feeding of the ticks as a result of severe tissue reaction. The experimental design did not permit proper collection of engorged ticks. Physical and clinical-pathological observations made followed the same pattern as for other regimes. The manifestations induced by this regime of infestation were quite severe, culminating in rapid deterioration in clinical condition and death in one and these are described as follows.

9.1. PHYSICAL (CLINICAL) SIGNS.

Immediate responses to attachment occurred in all calves soon after recovery from narcosis. However, at some of the infestations the reactions were suppressed at first but became accentuated later as the narcotic effects (analgesic) wore off completely. The intensity of reactions varied and at times calves appeared tolerant, but this was largely attributed to a big proportion of ticks wandering over hair for many hours before settling. The signs, as observed in other infestation regimes, were associated with pain and irritation. The dominant signs included restlessness, attempts to groom, frequent urination and increased salivation. Established tick feeding intensified the signs and elicited new ones in various systems as described below.

9.1.1. Rapid and serious deterioration in body condition commenced at the first infestation and worsened progressively in the subsequent exposures. The calves appeared wasted and hollow and their coats became ruffled. The demeanour and physique were similarly affected. Concurrently the calves became markedly weak, inert and were recumbent for most of the time. On standing the head and neck were held low and extended. The extreme weakness affected the intake of bulky feeds and eventually prostration developed in the last day or two.

9.1.2. The cardiovascular system was seriously affected as shown in the following parameters. Pyrexia levels of the mean rectal temperatures (p.m.) developed in all calves during the first three infestations but were reversed in the fourth exposure as animals were overwhelmed (Fig. 9.1). Response to the primary infestation under this regime combined features of the individual instar infestation regimes as illustrated in Fig. 9.2. The first temperature rise coincided with the engorgement of the larvae and nymphs and the latter peak was associated with engorgement of female ticks. The elevated mean rectal temperature of the compared uninfested controls during the first three to four days was of nonspecific origin. The sudden and remarkable change in the trend of the mean weekly pyrexia levels is amplified in Fig. 9.3 which illustrates the reaction to the third and fourth infestations. As expected an early pyrexia occurred during the third exposure but the temperature dropped suddenly before female engorgement was complete. Despite the massive stimulus during fourth exposure the temperature did not rise again to pyrexia levels. The phenomenon could be attributed to intoxication from massive infestation that culminated in ^acritical clinical state. The critical state was associated with markedly subdued physiological activity which influenced the temperature reaction. Thus deterioration in the blood picture (Figs. 9.4 to 9.6) and rapid prostration accelerated the response significantly. The depression in rectal temperatures

was terminally accompanied by poor vasomotor tone. In contrast the heart and pulse qualities remained practically stable with only slight intensification in rate and force on few occasions. Nonetheless, subdued activity was detected during prostration. The major changes in the visible mucous membranes affected the eyes mostly. Marked conjunctival congestion developed accompanied by persistent lachrymation which turned mucopurulent at times. These changes were more intense on the side(s) corresponding to the infested sites. Pallor developed in the calf that succumbed when the haematological levels became subnormal. Erythema developed consistently round attachment sites. Oedema appeared over ears and in other sites like eyelids and the maxillary and subparotid regions. Sometimes oedema was detected within six hours of attachment during repeated infestations. The acuteness of the responses could be partly attributed to relatively low age as the older calves infested with high density of adult R. appendiculatus manifested the severe effects much later in these systems.

9.1.3. The reaction of the lymph nodes draining the infested areas was minimal during the first infestation but became marked and persistent subsequently. Appreciable regression in size occurred after engorged females had detached and nodes remained small if subsequent infestation in the region was light. Nonetheless,

FIG. 9.1

Mean weekly Rectal Temperatures of three calves exposed weekly to successive concurrent three-instar infestations of Rhipicephalus appendiculatus compared with uninfested controls. Arrow marks critical normal temperature. (n) was reduced to two after 2nd infestation.

FIG. 9.2

Mean daily Rectal Temperatures of three calves exposed to first concurrent three-instar infestation of Rhipicephalus appendiculatus compared with uninfested controls. Arrow marks critical normal temperature.

FIG. 9.1

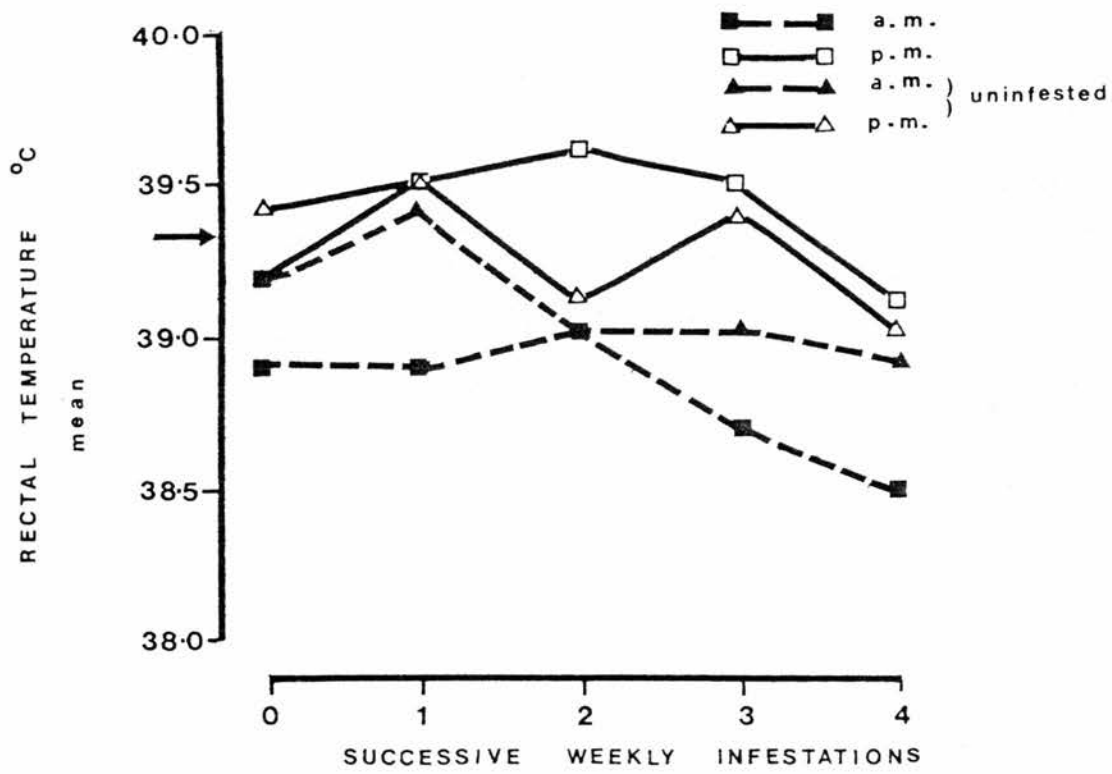


FIG. 9.2

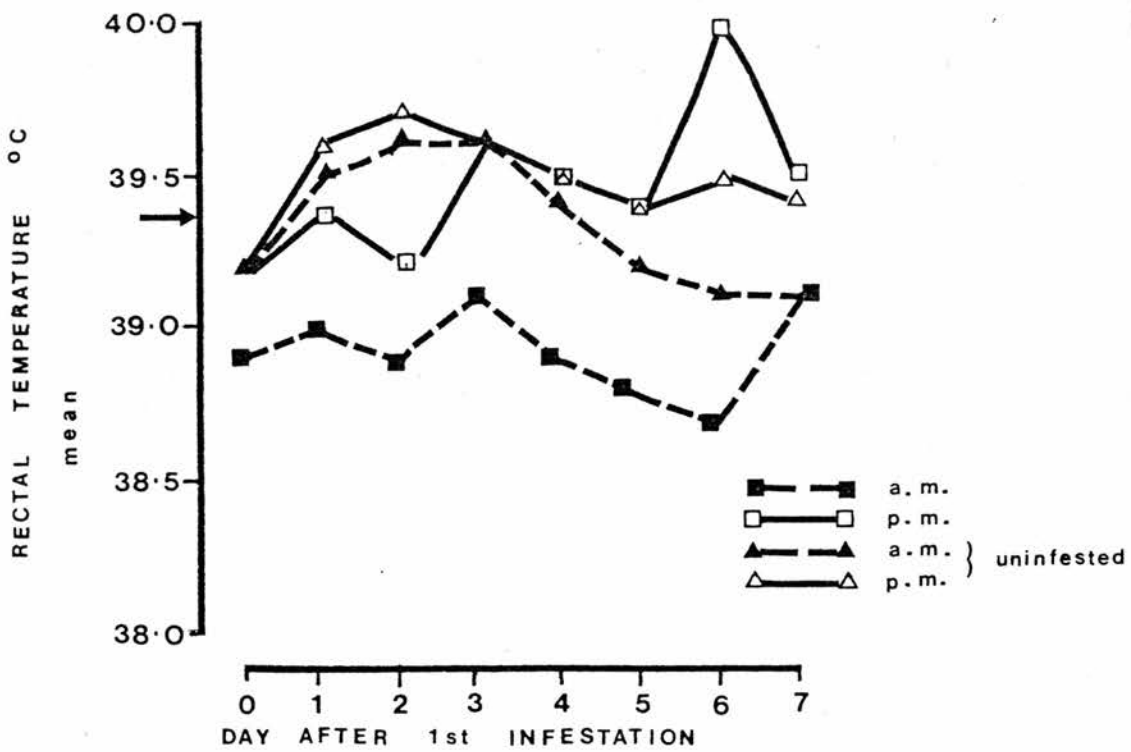


FIG. 9.3

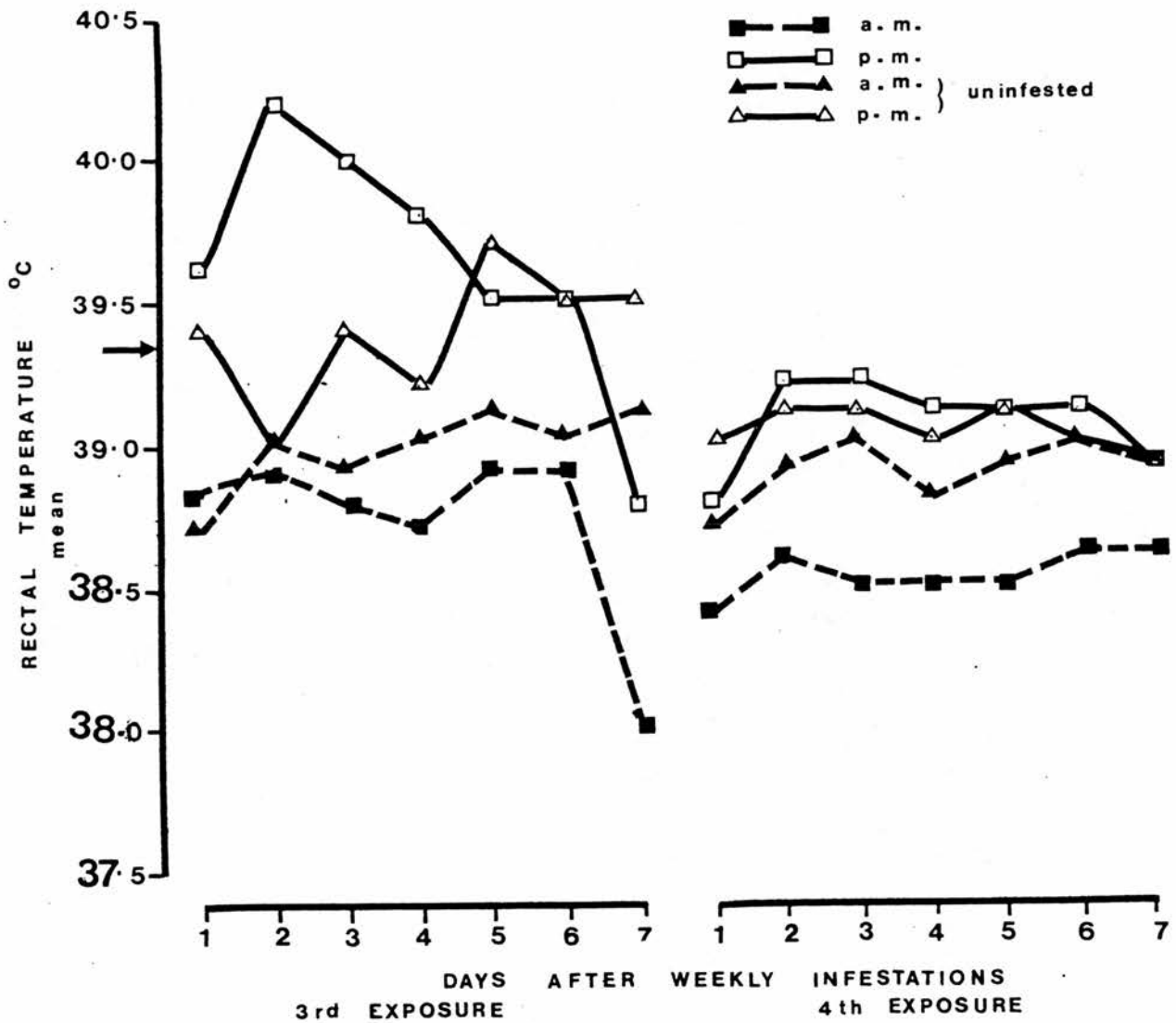


FIG. 9.3

Mean daily Rectal Temperatures of three calves exposed to third and fourth successive weekly concurrent three-instar infestations of Rhipicephalus appendiculatus compared with uninfested controls. Arrow marks critical normal temperature.

parotid nodes of two calves became grossly enlarged and failed to regress. Exploratory centesis drew sanguineous pus from one and sero-sanguineous lymph from the other. The sanguineous fluid indicates disturbance of the capillary endothelium leading to haemorrhage and congestion in the nodes. Comparative aspirations from nodes draining uninfested areas showed clear or slightly buff material. Thus the inflammatory changes are attributed to direct tick effects. The abscessed node eventually burst spontaneously releasing pus which was thick, yellow-brown and foul smelling. Bacteriology on the pus sample produced a pure culture of Corynebacterium pyogenes as obtained in C.5.1.4 & C.6.1.3. The calf was then withdrawn and slaughtered thus leaving only one standing as the second calf had succumbed. The respiratory system showed no significant changes but deep, forceful and stertorous breathing developed terminally in the succumbed calf. However, autopsies revealed inflammatory changes of the pulmonary tissue despite lack of clinical manifestations.

9.1.4. The digestive system functioned normally showing keen appetite but the succumbed calf fed progressively less from the second infestation onwards due to severe deterioration in physical condition. The calf passed very soft faeces which contained much mucus at times. The same calf manifested haemoglobinuria

(red-brown urine) for four days during the second infestation. As stated beforeⁱⁿ/(C.6.1.5), search of several blood smears made at various times did not reveal haematogenous parasites, e.g. Babesia sp.* Similarly the possible differential causes, e.g. leptospirosis, copper poisoning, did not conform to symptoms associated with the syndrome and would not be supported by the isolated nature of the case in a place inhabited by several animals. Haematuria causes were discounted because no erythrocytes were found in the urine. In view of these findings it can be asserted that the urinary problem was of an intra-renal disturbance

9.1.5. Pain and pruritis reactions represented the nervous system manifestations and were observed throughout the infestations. Some of the related signs have been described in (C.9.1.) and other infestation regimes (C.6.1). In contrast the pruritis effects were more widely manifested over the body but principally involved the ears, head and neck. The resultant grooming caused marked abrasions, exudation and encrustation. The intensity of pruritis was most severe during attachment and repletion phases of the ticks and since the immature instars engorged earlier this bridged the interval which would have occurred before the engorgement of the females. Thus the calves were continuously irritated until the detachment of engorged female ticks. The integumentary

* N.B. the plasma in the PCV tube was not discoloured.

system sustained severe injury mediated through direct and indirect effects of tick feeding as described under other infestation regimes of individual instars of R. appendiculatus (C.5.1.8). The more permanent effects associated with heavy clustering were primary inflammatory reactions round bite wounds and hypersensitivity reactions. Severe primary reactions culminated in alopecia, hyperkeratosis, and distortion of the pinna and the reactions were predominantly observed over the ears, neck, poll and withers and were compounded by grooming. Alopecia was also exacerbated by "scalding" from excessive exudation associated with abrasions and hypersensitivity reactions. Hypersensitivity manifested mild bleb formation principally over ears and occurred during secondary infestations. The lesions were discrete, 4.0 to 20.0mm diameter but ^{they}enlarged on coalescing. The skin of the succumbed calf lost elasticity terminally which was attributed to dehydration and emaciation.

9.2. CLINICAL-PATHOLOGICAL FINDINGS

9.2.1. Haematological estimations.

The mean absolute values and standard deviations of the erythrocytic indices and leucocytic series are shown in Figures 9.4 to 9.11. The mean values of the erythrocytic indices, except the mean corpuscular haemoglobin concentrations (MCHC), declined markedly to

FIG. 9.4

Means and standard deviations of Packed Cell
Volumes of three calves exposed weekly to successive
concurrent three-instar infestations of Rhipicephalus
appendiculatus.

FIG. 9.5

Means and standard deviations of Red Blood Cell
counts of three calves exposed weekly to successive
concurrent three-instar infestations of
Rhipicephalus appendiculatus.

FIG. 9.4

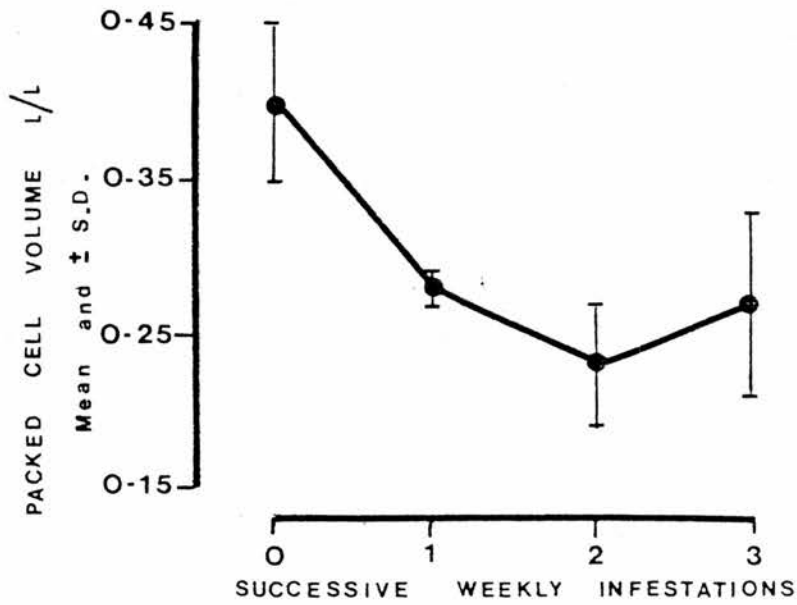


FIG. 9.5

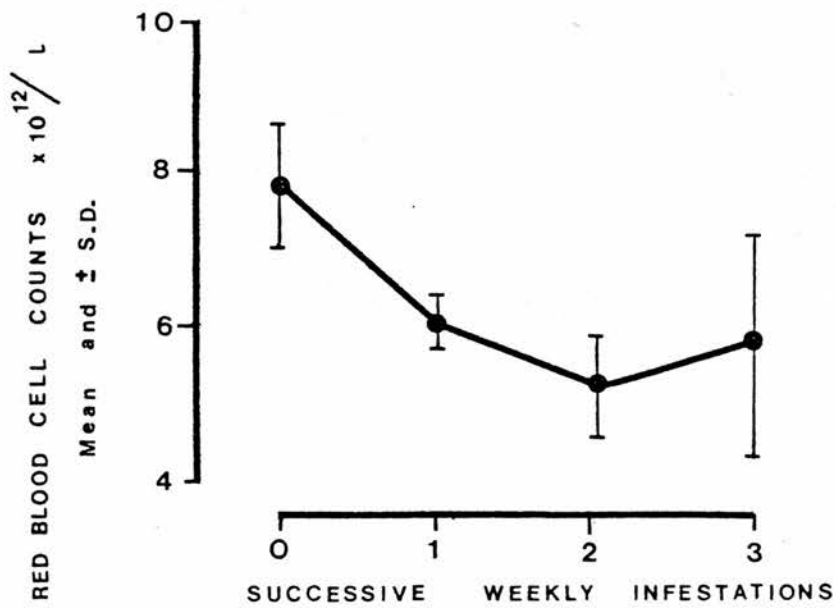


FIG. 9.6

Means and standard deviations of Haemoglobin
Concentrations of three calves exposed weekly to
successive concurrent three-instar infestations of
Rhipicephalus appendiculatus.

FIG. 9.7

Means and standard deviations of Mean Corpuscular
Volumes of three calves exposed weekly to successive
concurrent three-instar infestations of Rhipicephalus
appendiculatus.

FIG. 9-6

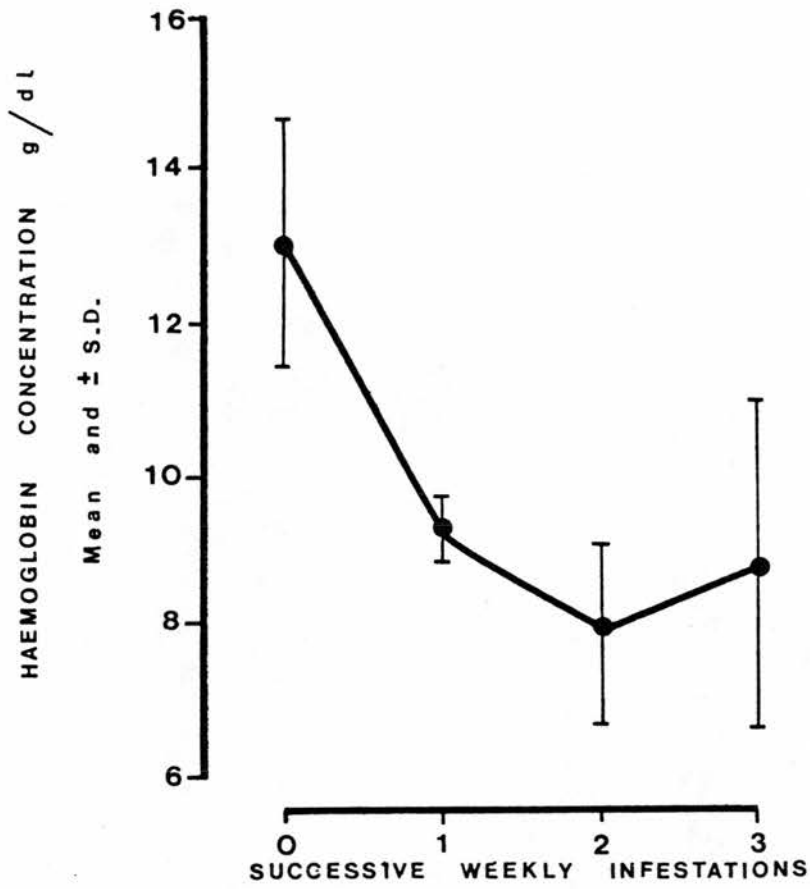
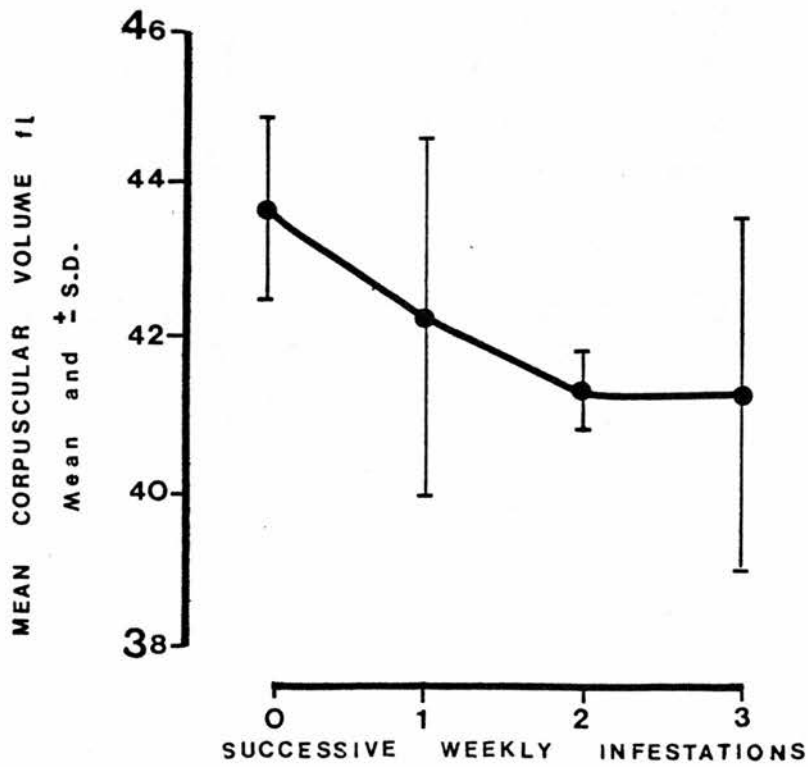


FIG. 9-7



subnormal levels during first two infestations. The slight recovery during the third infestation could be attributed to stimulation of the haemopoietic reserve as reflected in the increased standard deviations. However, mean values of the mean corpuscular volumes (MCV) and MCHC were within normal ranges. It is notable that the mean levels of the mean corpuscular volumes appeared stabilised and showed no corresponding response to that of the packed cell volumes, red blood cell counts and haemoglobin concentrations (HB). The stability in MCV levels would indicate that the sub-optimal levels attained in other indices were not critical to provoke corresponding response in MCV or the haemopoietic system was inhibited by the effects of tick secretions. The reverse trend in the mean corpuscular haemoglobin concentrations (MCHC) levels cannot be explained relative to the levels of red blood cell counts and haemoglobin concentrations (Figs. 9.5, 9.6). Similarly the rapid decline in the erythrocytic indices could be attributed partly to age susceptibility. The changes in the erythrocytic indices would suggest the development of a microcytic hypochromic anaemia.

The mean values of the leucocytic series showed similar trends within normal ranges but the lowest mean levels which appeared in the first infestation were followed by appreciable rises (Figs. 9.9 to 9.11). The sharp rise in the mean levels of absolute neutrophil counts, which contributed significantly to the total white blood cell count, could be associated with secondary

FIG. 9.8

Means and standard deviations of Mean Corpuscular Haemoglobin Concentrations of three calves exposed weekly to successive concurrent three-instar infestations of Rhipicephalus appendiculatus.

FIG. 9.9

Means and standard deviations of White Blood Cell counts of three calves exposed weekly to successive concurrent three-instar infestations of Rhipicephalus appendiculatus.

FIG. 9-8

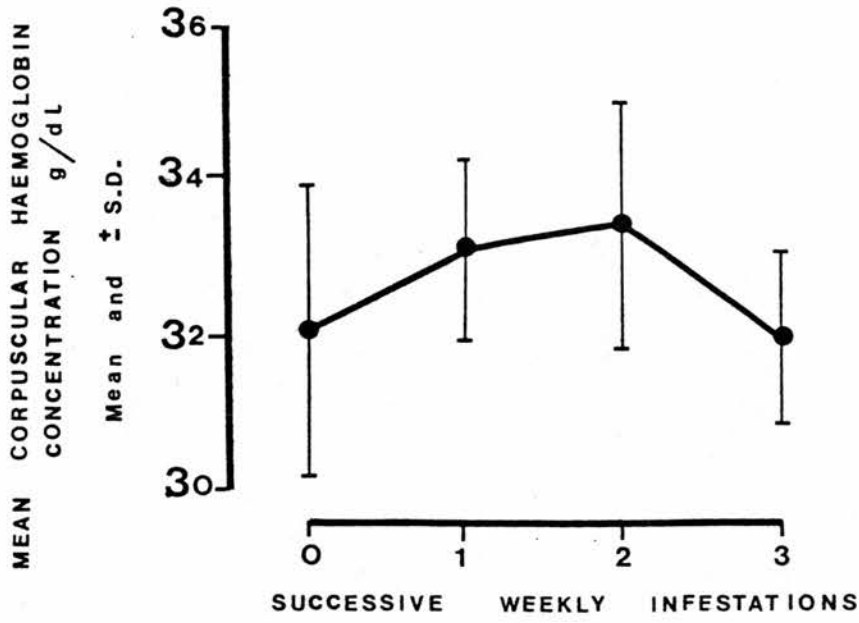


FIG. 9-9

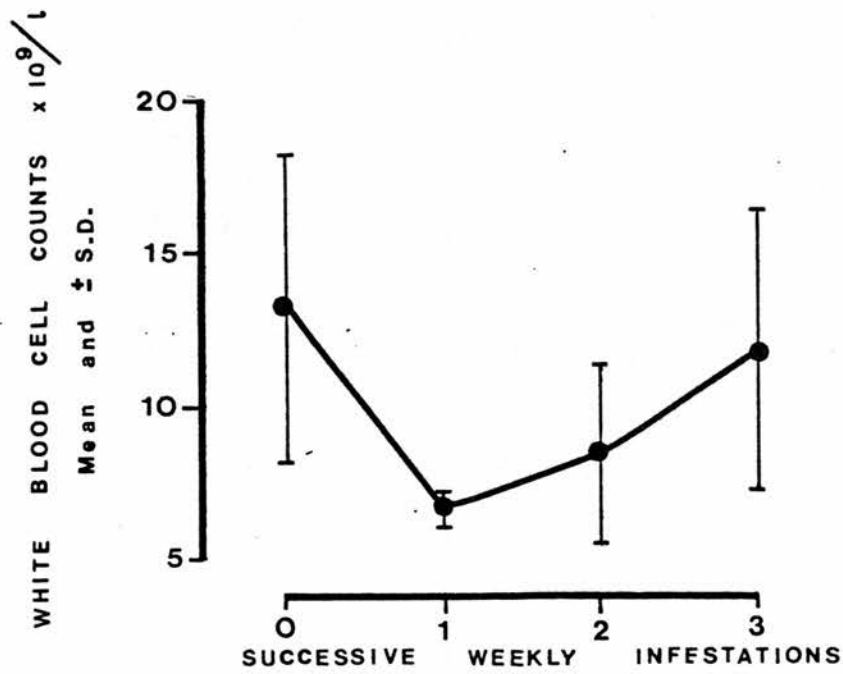


FIG. 9.10

Means and standard deviations of absolute Neutrophil Cell and Eosinophil Cell counts of three calves exposed weekly to successive concurrent three-instar infestations of Rhipicephalus appendiculatus. ((0) for Eosinophils).

FIG. 9.11

Means and standard deviations of absolute Lymphocyte Cell counts of three calves exposed weekly to successive concurrent three-instar infestations of Rhipicephalus appendiculatus.

FIG. 9-10

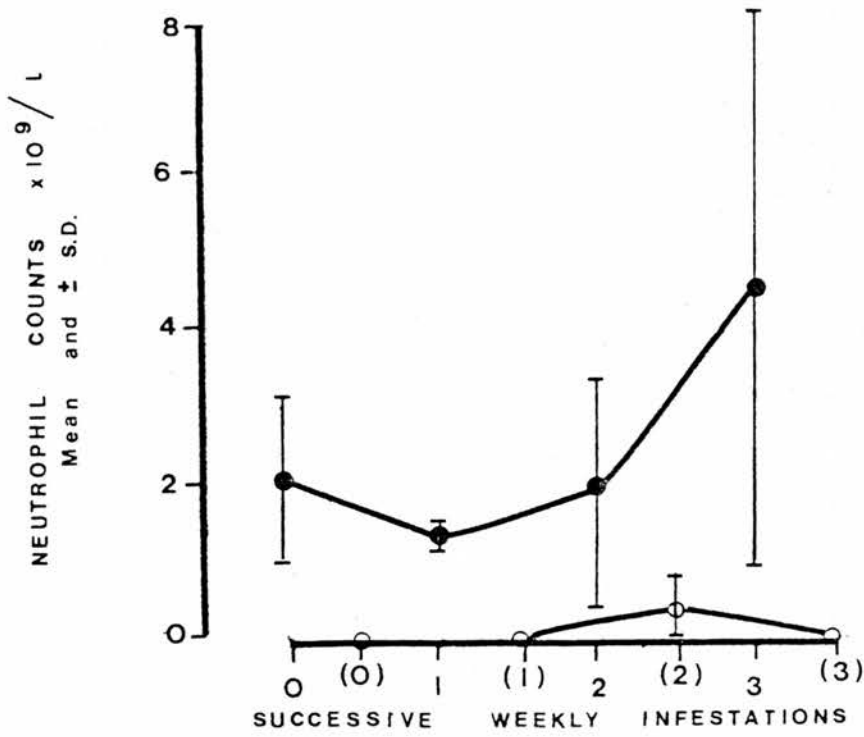
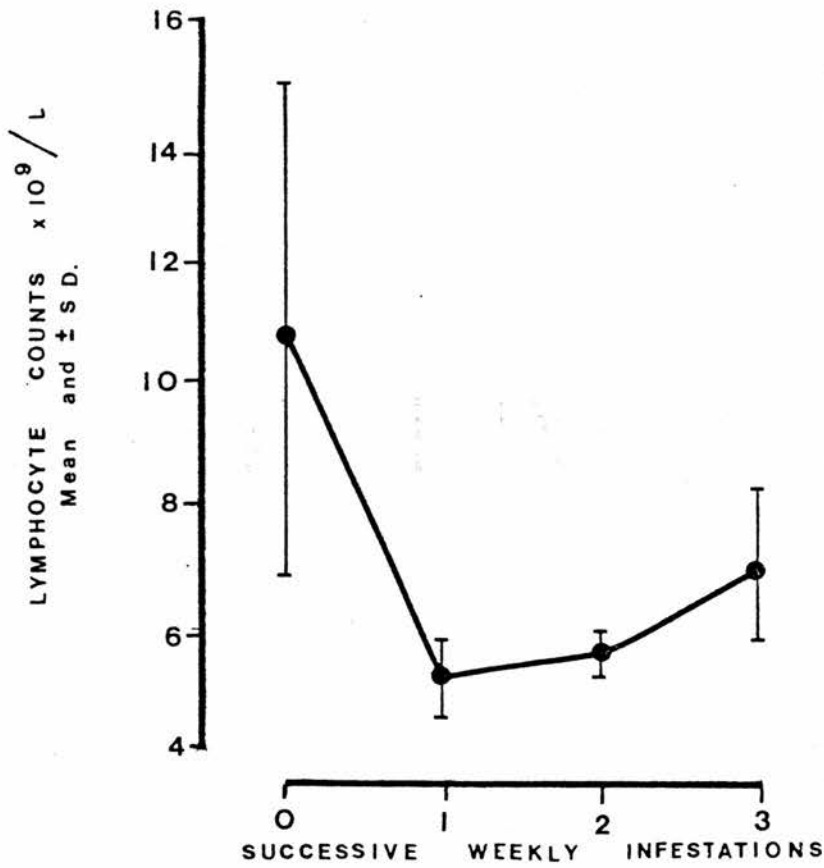


FIG. 9-11



complication e.g. abscessation. The eosinophils in the peripheral circulation were scarce (Fig. 9.10) and the reason could be as explained before (C.5.2.3.).

The rise in eosinophils and neutrophils may be agonal discharge from the bone marrow in a dying animal.

The big standard deviations of the pre-infestation counts of total white blood cell counts and absolute lymphocyte counts could be attributed to age (O'Kelly, 1974, Schalm et al., 1975).

9.2.2. The analysis of variance as described in C.4.2. revealed significant differences between the uninfested control group (I) and other experimental groups, including this group (VI), in all estimated parameters (Tables 4.1 to 4.8), except the neutrophils and eosinophils (Tables 4.7 & 4.9), during the first four successive estimations. All significant differences, except on one occasion, were revealed during infestations which indicates induced changes. The frequency and levels of significance are described in C.4.3.2.

Duncan's New Multiple Range test (DNMRT) thus resolved significant subsets in which this group (VI)

responded similarly to other groups and also overlapped once between subsets (Tables 4.10 to 4.16). The group did not attain the highest mean values, except once it appeared within the subset ranked highest in the analyses of mean corpuscular volumes (Tables 4.13). In contradistinction the test ranked the group lowest on six out of eleven occasions during infestations. This

proportion clearly indicates the group was markedly affected compared with others exposed to individual instar infestations of R. appendiculatus. The test that further revealed/the frequency of closest association in responses of erythrocytic indices was highest with group (IV) (infested with nymphs) followed very closely by group (III) (infested by high density adults) and no association was found with groups (V) (infested with larvae), (II) (infested fortnightly with low density adults) and (I) (uninfested controls). The remoteness in association with latter groups could be attributed to a much earlier decline in the responses of the indices of this group (VI). Thus the group contrasted significantly with the controls. The association with group (IV) could be attributed to more successful nymphal feeding in the initial stages of exposures (Fig. 3.3) which caused early alterations in the indices, and delayed development of drastic haematological changes in group (III). As a reminder the estimations for this group were only done up to the third infestation.

9.2.3. Biochemical estimations

The mean values and standard deviations of serum protein levels (S.P.) are presented in Fig. 9.12 and the only mean values and standard deviations of immunoglobulins (IG) and serum glucose (S.G.) from pre-infestation estimations were 29.3 ± 7.35 (Turbidity

FIG. 9-12

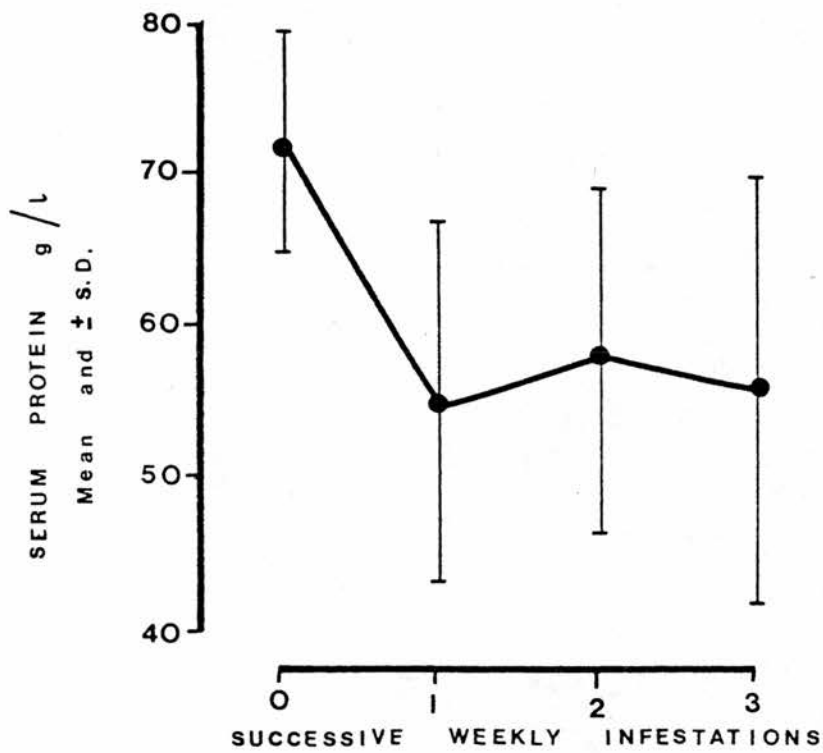


FIG. 9.12

Means and standard deviations of Serum Protein levels of three calves exposed weekly to successive concurrent three-instar infestations of Rhipicephalus appendiculatus.

units, T.U.) and 3.15 ± 0.35 (mmol/l) respectively.

The mean values of serum protein dropped sharply to suboptimal levels and also showed big standard deviations. Thus hypoproteinaemia concurrent with marked decline in erythrocytic indices developed in the calf that succumbed. Hypoproteinaemia could be attributed to blood loss, inefficient feed utilisation and excessive tissue wasting.

9.2.4. Analysis of variance revealed significant differences between the uninfested control group and other experimental groups, including this group (VI) in the three parameters during pre-infestation estimations and subsequently in serum protein levels (Tables 4.18 to 4.20).

The Duncan's New Multiple Range test similarly revealed significant subsets in which the group responded similarly to other groups and also overlapped between subsets (Tables 4.21 to 4.23). The group (VI) assumed the lowest ranking in serum protein during the third infestation and appeared again in the lowest subset during second infestation. During these two infestations the group was closely associated with groups (I) (controls) and (V) (larval infestation). The association is coincidental as low serum protein levels appeared in the other two groups which were not stressed at the time.

9.3. AUTOPSIES

Two calves were autopsied, one (hereafter A) died naturally and the second (B) was slaughtered on withdrawal because of lymph node abscessation. The significant gross pathological changes in the various systems were as follows. The integument lesions associated with reactions to tick feeding appeared as rough tarnished coat, pustules, alopecia and hyperkeratosis the carcass condition revealed moderate emaciation (B) and severe cachexia (A). The visible mucosae were extremely pale (A); and in (B) conjunctival vessels were congested and eyes sunken (dehydration). There was considerable wasting of muscles which were pale, loss of adipose tissue in both cases but dropsical changes also appeared in (A). Superficial lymph nodes were enlarged, haemorrhagic, and on section some showed **marked** necrosis and suppuration in the medulla (A & B). An abscess in parotid node was found in (B). The lymph node changes corroborate the observation made by van Rensburg (1959), Thomas & Neitz (1958) on degenerative changes in lymph nodes of steers predominantly infested with R. appendiculatus.

The spleens were moderately and markedly reduced in size for (B) and (A) respectively and the cut surface revealed predominance of white pulp (A). Liver changes were not significant though (A) showed a slaty colour. The kidneys had a dark subcapsular surface and

brown cortical surface (A & B) whilst congested calyces and white gelatinous infiltration in the pelvis appeared in (A). The congestion contrasted the general pallor in the rest of carcass (A). The gelatinous infiltration was also found in calves exposed to high density infestations of adult R. appendiculatus. However, it is not clear whether the congestion was connected with the haemoglobinuria

The thorax showed slight straw coloured hydrothorax. The heart showed pneumo- and hydropericardium, no coronary fat, white epicardial striations and flabby myocardium. The tricuspid valve carried 5mm red nodules (A). Red hepatization of right apical lobes of the lung (A & B) and froth in bronchioles (A) were found. The joints of (B) were infiltrated with gelatinous material which was suggestive of arthritis though no clinical evidence was detected. It is possible that the abscessation produced a pyaemia which localised in the joints, setting up a low grade arthritis. In support of this suggestion, Pirie (1979) states that C. pyogenes has been associated with a fatal septic arthritis in calves. The bone marrow for (A) was of red gelatinous consistency.

In conclusion the autopsies revealed syndromes including emaciation, gelatinous infiltration in joints and kidneys, circulatory derangement related to anaemia and haemorrhage, lymphadenopathy confounded with abscessation and pneumonic changes. These reactions are attributable to a combined effect of exsanguination, intoxication and secondary infections.

9.4. SUMMARY OF SIGNIFICANT FINDINGS

The successive infestations of concurrent three instars of Rhipicephalus appendiculatus severely disturbed the health of the calves. The severe effects developed rapidly and culminated in death and secondary infections. Thus the ticks applied provoked severe loss in body condition, lymphadenopathy, serious direct and indirect integumentary reactions, inhibition of physiological activities, e.g. circulatory function. The severity of manifestations in the different systems varied. The immediate response to tick attachment evinced restlessness, grooming and excessive secretions from visible mucous membranes and these were associated with pain and irritation. The clinical loss in body condition culminated in emaciation, debility, severe depression and prostration. The induced pyrexia suddenly merged into suboptimal temperature levels indicative of inhibition of the heat regulating mechanism despite the continued stimulus of high density of feeding ticks. There was pallor or congestion and excessive secretions in the mucosae. Oedema and erythema developed in the infested areas. Severe and persistent lymphadenopathy was induced and was confounded by Corynebacterium pyogenes abscessation. Appetite, though reduced, was maintained. Haemoglobinuria of undetermined origin was observed. Primary dermatitis from tick feeding was confounded by hypersensitivity bleb formation and

grooming provoked by pruritis sensation. Pruritis was continuous throughout the individual infestations. Sequelae to infestation were alopecia and hyperkeratosis, especially over ears. Clinical-pathological indices presented suboptimal levels during infestation. Bone marrow activity persisted though ^{the} haemopoietic response was inhibited in the latter stages. The group (VI) shared significant differences and similarities with other designated experimental groups in the clinical pathological estimations.

The autopsies revealed significant changes in the integument, carcass state, lymph nodes, spleen, kidneys, heart and lungs. Some of these changes confirmed the clinical manifestations whilst others could not be explained. Thus the carcass showed pallor, wasting and dropsical degeneration and ^{the} lymph nodes were haemorrhagic, necrotised and abscessed. The pathogenesis of congestion and gelatinous infiltration of kidneys and joint infiltration was not clear. Gelatinous consistency and light red colour of the bone marrow indicated anaemia and debility.

Thus the infestation regime induced deprivation through reduced feed intake and conversion and rapid breakdown of body tissues associated with derangement in physiological processes and secondary complications. The immune responses, represented by hypersensitivity reactions and pruritis can cause serious injury in the host. The rapid deterioration in clinical state

could not be wholly attributed to the simple tick feeding process. The effects of secondary complications should be considered. Sudden suppression of the pyrexia reaction and inhibition in haemopoietic response terminally indicated intoxication effect. The cause of death could then be attributed to anaemia, debility and intoxication. The concurrent application of all three instars of R. appendiculatus and the relatively young age of the calves contributed greatly to the acuteness of the calf reactions.

CHAPTER 10

EXPERIMENT (VII): RESPONSES OF CALVES AND GUINEA PIGS TO THE THREE INSTARS, APPLIED INDIVIDUALLY, AND SALIVARY GLAND EXTRACT (SGE) OF RHIPICEPHALUS APPENDICULATUS DURING SECONDARY EXPERIMENTS.

10.1. Experiment (VII) comprised four sub experiments (VII.I) to (VII.4) designated secondary and based on null hypotheses stated later under appropriate sections. The host range was extended to include guinea pigs besides calves, which were the only host used in the primary experiments. The original theme centred on the investigation ^{the} of/effects of tick agent(s) in cattle but a number of constraints appeared and this necessitated the use of the guinea pig as a model. The constraints included finance for purchase of calves and their feeds, sufficient accommodation for the required numbers, limited laboratory facilities to handle frequent samples. The use of the guinea pig solved most of these requirements and had the advantage that the species is extensively used for studies on immune responses to tick effects under laboratory conditions. The cheap price and small size allowed for replicates in trials and, overall, placed much less demand on tick supplies. The time factor was another constraint in the execution of the experiments and thus the investigations had to be kept as simple as possible. Hence a drastic reduction in the analysis of laboratory material

was decided. The use of the guinea pig was further justified by aiming at demonstrating whether Rhipicephalus appendiculatus secretions cannot induce discernible responses in other exposed hosts.

The primary experiments revealed diverse responses in calves exposed repeatedly to all three instars of R. appendiculatus. The instars were applied individually and concurrently to separate calves. The responses induced varied from inapparent/mild to severe local or systemic reactions and in extreme cases culminated in cachexia and death. Significant clinical-pathological reactions included dermatitis, hypersensitivity state, pyrexia, lymphadenopathy, depressed blood composition, extreme loss of condition and weakness. Out of the five regimes applied, the individual adult and concurrent three instar infestations of R. appendiculatus were the most harmful. The deaths recorded occurred in these regimes. Thus the Rhipicephalus appendiculatus, at the densities applied, caused debility and death in the calves. In order to test these findings the secondary experiments were conceived and aimed at confirming and demonstrating the inability of the agent(s) in the salivary secretion of R. appendiculatus to induce some of these observed responses. The possible responses tested included reaction to primary exposure, effects of passive humoral antibodies, distinction between responses to primary and repeated (secondary) exposures in individual hosts,

and influence of antihistamines on the reactions.

The effects of the agent(s) were mediated through tick feeding and inoculation of female tick salivary gland extract (hereafter called SGE) and homologous immune serum from hosts exposed at least two times to the agent(s). Results from the primary experiments indicated that an immune response was induced during infestations of R. appendiculatus. The related responses affected tick performance and manifested as clinical hypersensitivity reaction of blebs and urticarial eruptions. Hence, investigations of the hypotheses included tests on the passive transfer of immune serum. Similarly, circulatory disturbances and pruritis were of a nature suggestive of pharmacological activity of histamine/allied substances released during interaction between tick secretions and host tissues. The pharmacological activity was thus tested with antihistamines administered to passively and actively sensitized hosts. The procedures used for each hypothesis will be further mentioned under the relevant sections. However, the guinea pigs were reared conventionally and kept free of ticks until they were used (C.2.2.2.). Different levels of infestations with individual instars of R. appendiculatus for both hosts and SGE for guinea pigs were used to demonstrate local/systemic reactions. The cutaneous Arthus reaction was tested with SGE (antigen). Deliberate massive infestations were applied to guinea

pigs to demonstrate whether the agent(s) in the tick secretion can induce lethal effects. Animals were examined daily and minimal sampling carried out during exposures. The few haematological samples collected served just as screening estimations of responses. As for primary experiments, autopsies were performed on dead hosts. The results obtained from the investigations on the different hypotheses were as follows.

10.2. EXPERIMENT (VII.1): RESPONSES TO HYPOTHESIS (I).

The hypothesis stated, 'The agent(s) cannot provoke a clinical-pathological response on primary exposure'. As stated above, the first exposures during primary experiments provoked severe tangible or inapparent clinical manifestations. Verification of these findings sought for definite signs in the secondary experiments where hosts were exposed to low and high levels of the tick agent(s). Nonetheless, calves with inapparent manifestations to first infestations developed more specific and tangible reactions on exposure to additional infestations. This was attributed to immunological sensitization: thus development of antibodies to agent(s) of R. appendiculatus was assumed for secondary experiments. Demonstration of such immune responses was effected by cutaneous Arthus reaction test. The earliest stage for the challenge was influenced by studies on Boophilus microplus infestation in cattle made by Roberts (1968a). The design followed in the investigations on the hypothesis is summarised in Table 10.1.

TABLE 10.1.

SUMMARY OF EXPERIMENTAL DESIGN FOR EXPERIMENT (V11.1).

HOST/NUMBERS	SGE+	TREATMENT A	APPLIED N	L	CA
Guinea pigs					
9	*	3 to 5 x 0.1ml @ S.C./i.d.			
10	-	* 60 to 800 ear/trunk (male/female)			
2		700 & 5000* trunk			
3				2000* to 20000	
<u>12</u> <u>24</u>	including +			tested with SGE*	
Calves					
6		300* to 1000 ear, male/female			
2		4000* & 5000 ear			
2		10000* & 50000 ear.			
<u>6</u> <u>10</u>		tested with larval & SGE*			

SGE = salivary gland extract; A = adults; N = nymphs;

L = larvae; CA = cutaneous Arthus reaction

* under treatment applied.

24 = total number treated and 12 included the 9 guinea
pigs given SGE.

TABLE 10.2.

SUMMARY OF RESPONSE OF CALVES TO FIRST EXPOSURE TO THE AGENT(S) OF R. APPENDICULATUS.

Parameter Affected	Adults	SOURCE OF AGENT(S) Nymphs	Larvae
B/C	(+)	NAD	NAD
CVS:			
Erythema	++	+	++
Oedema	+	+	+
Mucosae	Congestion and lachrymation		
Pyrexia	+	NAD	NAD
L/N	++	+	NAD
Pruritis	NAD	+	+
Integument	Eczema, exudation, necrosis, papules & transformation.		
CA	Immediate +++ to ++++ 10 to 14 dpi.		

(+) very slight, + slight, ++ & +++ moderate, ++++ severe,

NAD - no abnormality detected, CVS = cardiovascular system, L/N = lymphadenopathy, CA - cutaneous Arthus reaction, B/C = body condition.

The densities of individual instars of R. appendiculatus applied varied and the dosage rates of the salivary gland extract (SGE) ranged between 3 to 5 x 0.1 ml for individual guinea pigs. Tick performance was observed as before but weights were not taken. Similarly physical examination followed the same procedure as for primary experiments, but the haematological samples were minimal and biochemical estimations were not done. Some of the hosts were prophylactically dosed with antibiotics to control possible secondary complications. The C.A. test was administered between ten and fourteen days after commencement of exposure. Standard procedures for the test were followed and the readings taken included erythema, oedema and skin fold thickness at the site. The clinical-pathological responses produced are summarised in Tables 10.2, 10.3, i & ii. The temperature reactions are presented in Figs. 10.1 & 10.2. Eight guinea pigs died during or shortly after tick exposure and the gross pathological changes were recorded.

10.2.1. Generally a slight decline was observed in body condition of calves, especially under adult tick exposure. The guinea pigs on the other hand were severely affected, e.g. two guinea pigs exposed to adult instar lost an average 200gm each in twelve days. Nevertheless, when death occurred rapidly the carcass condition of the guinea pigs remained good. Guinea pigs became depressed and barely managed to move, thus the condition

TABLE 10.3.i.i.

SUMMARY OF RESPONSE OF GUINEA PIGS TO FIRST EXPOSURE TO THE AGENT(S) OF R. APPENDICULATUS

PARAMETER AFFECTED	Adults	SOURCE OF Nymphs	AGENT(S)	Larvae	SGE
B/C	++++	+++		NAD	NAD
D/P	+ to +++ & Coma	depression, weakness, prostration			NAD
CVS: Erythema (skin)	++ & cyanosis or pallor	++		++	NAD
Mucosae	+ congestion or pallor, discharges				NAD
Pyrexia	NAD/ S/N/+ to +++				-do-
Blood Smear.	Watery blood: reticulocytes, eosinophilia				-do-
Respiratory	Dyspnoea under anaemia crisis				-do-

+ slight, ++ & +++ moderate, ++++ severe; NAD = No abnormality detected; D/P = demeanour/physique;
 CVS = cardiovascular system; S/N = subnormal; GLT = digestive system;
 +ve = positive. B/C = body condition.

TABLE 10.3.ii
SUMMARY OF RESPONSE OF GUINEA PIGS TO FIRST EXPOSURE TO THE AGENT(S) OF R. APPENDICULATUS.

PARAMETER AFFECTED	Adults	SOURCE Nymphs	AGENT(S)	Larvae	SGE
GLT	Capricious appetite, anorexia, constipation or diarrhoea				NAD
Urinary	Oligouria terminally				-do-
Pruritis	Impulsive but +++, traumatization				-do-
Integument	+ Exudation, latent bleeding, red spots				Necrosis & induration
CA	+ve	+ve		+ve	+ve
Death	+ve	+ve		+ve	NAD

+ slight, ++ & +++ moderate, NAD = No abnormality detected; GLT = digestive system; CA = cutaneous Arthus reaction.
+ve = positive.

FIG. 10.1

Mean daily Rectal Temperatures of guinea pigs exposed to primary infestation of adult Rhipicephalus appendiculatus compared with uninfested controls. (Arrow marks arbitrary critical normal temperature).

FIG. 10.2

Mean daily Rectal Temperatures of calves exposed to primary infestation of adult Rhipicephalus appendiculatus compared with uninfested controls. (Arrow marks critical normal temperature).

FIG. 10-1

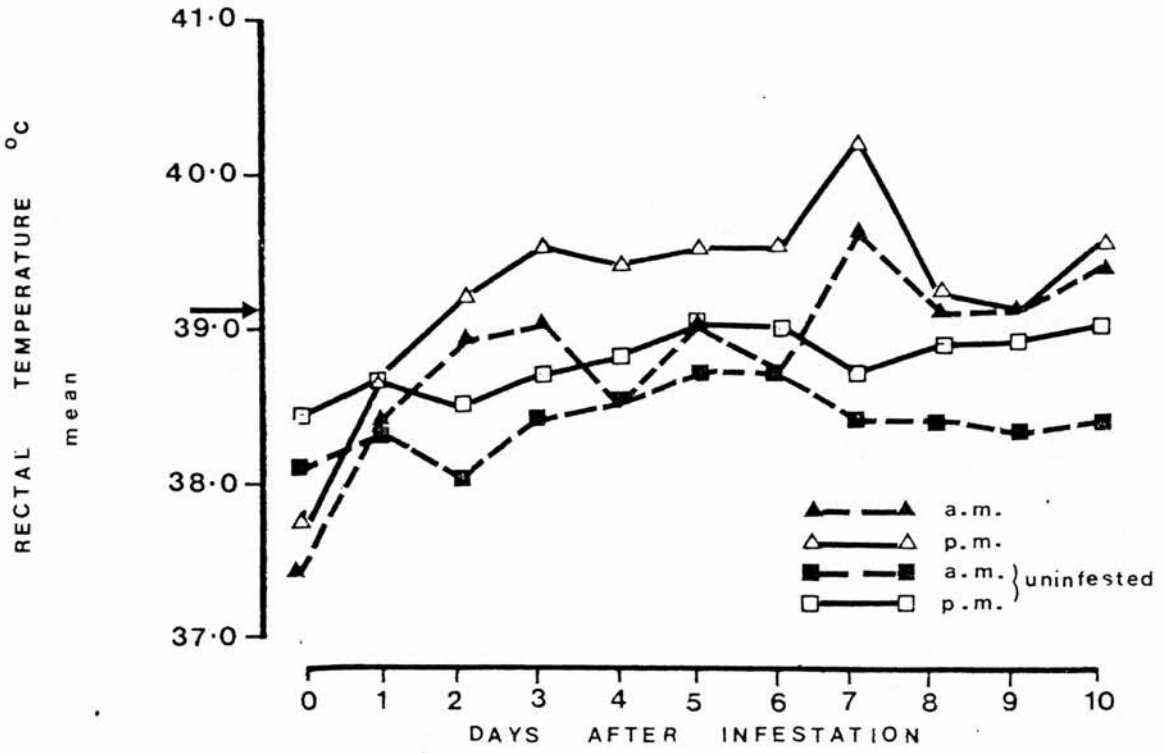


FIG. 10-2

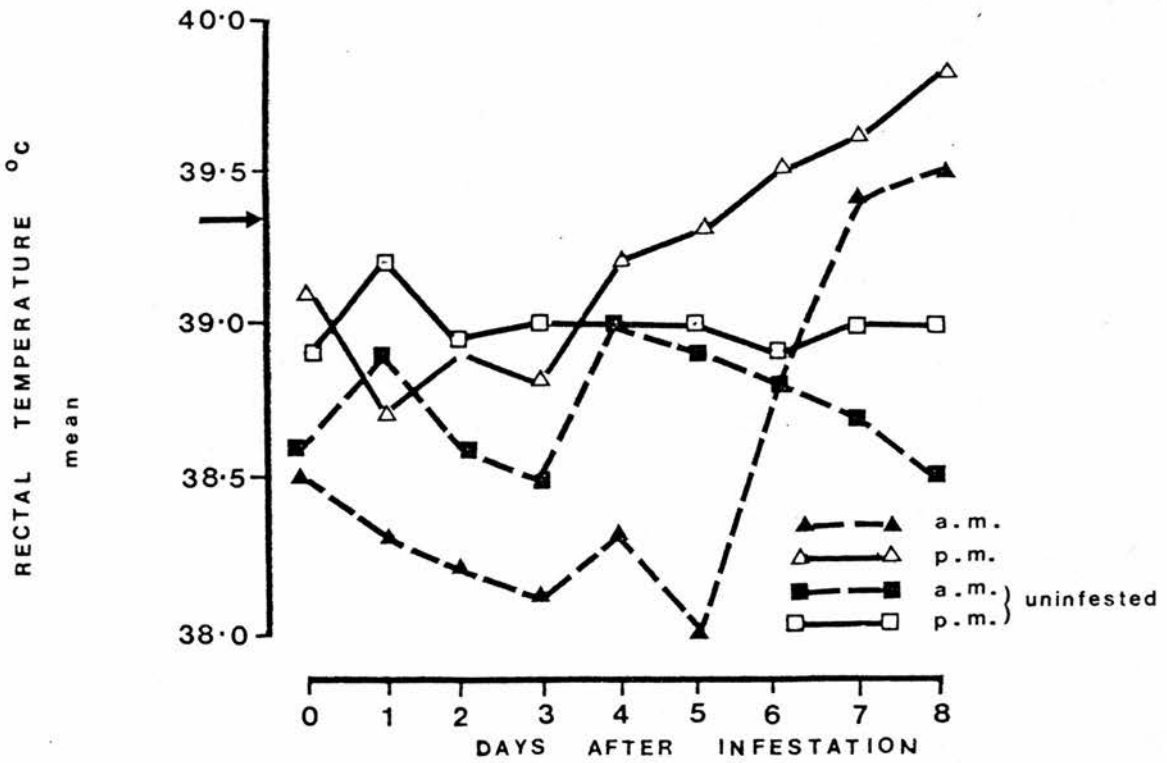
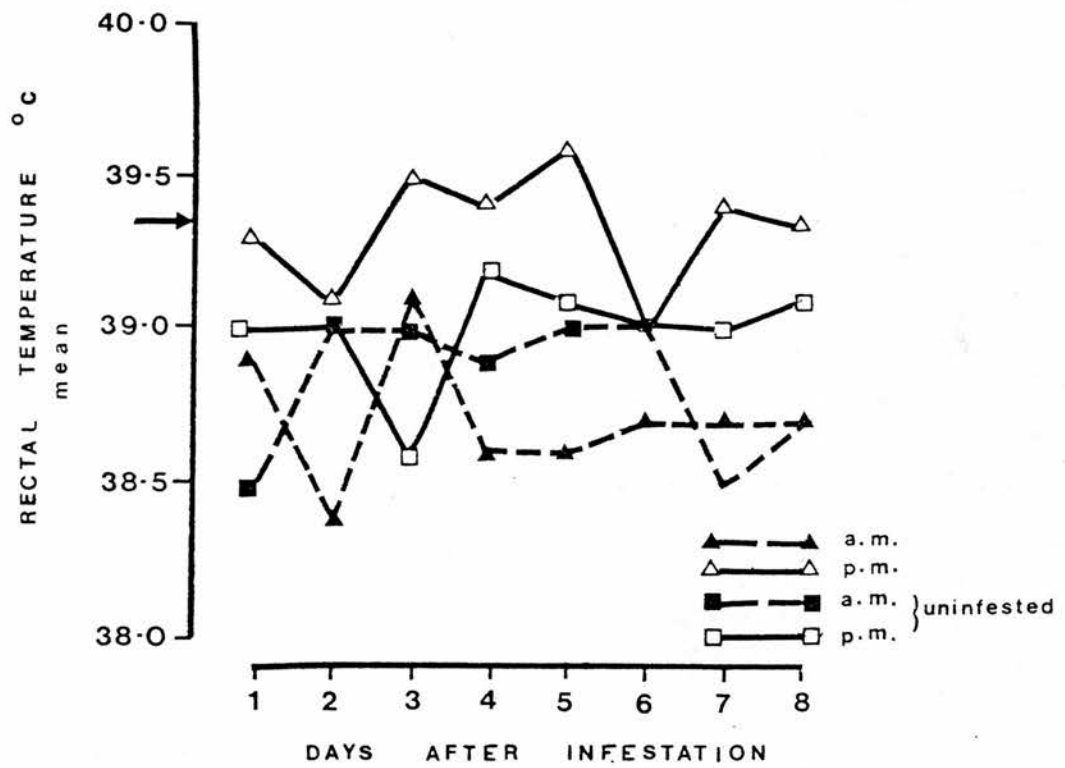


FIG. 10.3

Mean daily Rectal Temperatures of calves exposed to repeated infestation of adult Rhipicephalus appendiculatus compared with uninfested controls. (Arrow marks critical normal temperature).

FIG. 10-3



culminated in prostration, and coma before death. However, exposure to low densities of R. appendiculatus infestation did not induce significant disturbance in condition of some guinea pigs. The responses evinced by calves conformed to results of primary experiments but the very severe reactions of the guinea pigs were comparable to effects demonstrated by Theiler (1921) in a horse and heifer exposed to massive Boophilus decoloratus infestation. The effects were predominantly associated with circulatory disturbances which were manifested as follows in these investigations. Erythema of the skin developed round attachment sites in both hosts but merged into cyanosis or pallor terminally in guinea pigs which succumbed. Cyanosis, which also appeared at tick engorgement in some animals, indicated poor oxygenation of blood in the skin. Oedema was evident on^{the} ears of calves and the affected tissues became tender. Ocular changes in both hosts showed varied conjunctival congestion and lachrymation. However, the eye(s) on the infested side(s) showed^a/more intense reaction in calves whilst such distinction was not apparent in guinea pigs. Cyanosis and pallor in the visible mucous membranes similarly appeared in succumbed guinea pigs. These terminal changes were attributable to toxæmia and anaemia (exsanguination). The cyanosis could have also arisen from circulatory failure causing reduced oxygen tension in peripheral tissues. Guinea pigs also produced^a/mucoïd discharge which partially

encrusted the nostrils. Pyrexia was ^{induced} in both hosts. Response of a mild degree appeared only in some of the calves exposed to adult instar agent(s). The majority of guinea pigs in contrast reacted to all three instars and their response is represented in Fig. 10.1 which demonstrates reaction to adult instar. The levels of the pyrexia ranged from slight to marked (the temperatures in the uninfested controls were 37.4° to 39.0°C). In both hosts the peak of the pyrexia reaction coincided ^{the} with/engorgement period. The lack of pyrexia in calves exposed to larval and nymphal infestations was predictable ^{the} from/results of primary experiments. In fatal cases the rectal temperature of guinea pigs became subnormal for two to four days before death. However, during overwhelming massive exposure the normal temperature of the guinea pigs suddenly became subnormal without rising at all. Such animals died in two to three days of exposure. The dramatic depression suggests paralysis of the heat regulating mechanism which could be attributable to intoxication. Severe anaemia could be a contributory factor but there was no evidence of that in these cases. Thus reactions described above confirm the responses in primary experiments though the guinea pigs evinced extreme reactions which culminated in some deaths.

10.2.2. The lymphatics showed enlargement up to 2 x the normal size in parotid nodes of calves, being most prominent in exposure to adult R. appendiculatus. Assessment of change in sizes was not done in the guinea pigs because

of anatomical limitations but relative sizes were derived from autopsies on the dead and ones bled out for normal serum. The responses in calves confirmed the observations made during primary experiments. Respiratory function was generally unaffected, except for few guinea pigs which developed dyspnoea associated with anaemia crisis. The dyspnoea showed a pattern of very deep, forceful and irregular breathing. This type of respiration was characteristic of the "thumps"^{*} described in piglet anaemia. Thus the dyspnoea observed in guinea pigs was at variance with the reactions recorded in calves during the primary experiments. However, such critical signs were predictable under massive tick exposure and further demonstrated that such exposures cause rapid loss of blood. The digestive functions were markedly affected in guinea pigs but not in calves. Appetite was capricious and the digestive tract activity became generally depressed and terminally constipation or diarrhoea appeared. The constipation (failure to pass faeces) was associated with general emptiness revealed at autopsy on some guinea pigs which had no ingesta in almost the entire gut. Where ingesta was found the faecal pellets were quite dry, hard and coated with tenaceous mucus. The appearance of diarrhoea cannot be explained as autopsy revealed no severe enteritic changes which could be incriminated. Constipation could also have been caused by dehydration or bowel stasis from pyrexia or intoxication. A proportion of these animals would not drink when signs of

* N.B. (Hungerford, 1975).

depression developed. Thus the responses demonstrated that massive exposure to agent(s) of R. appendiculatus can severely depress the digestive functions leading to deprivation. The urinary system was similarly affected and the guinea pigs terminally developed oligouria. Autopsy revealed urine retention or^a/virtually empty urinary bladder in different guinea pigs. Lack of urine would mean severe dehydration, associated with adipsia or renal failure. In a proportion of such cases kidneys were haemorrhagic and enlarged, reactions that can restrict urine excretion.

Irritability, associated with pruritis, appeared during the engorgement period of larvae and nymphs in calves. The calves became restless, shook their ears/head and showed an urge to groom. On the other hand guinea pigs manifested impulsive irritability to tick attachment, thus confounding the development of pruritis attributable to tick effects. The development of pruritis in calves differed from observations made in primary experiments where the reaction occurred first in animals exposed to repeated infestations of adult R. appendiculatus. The explanation would be the relatively very low densities of adults applied accompanied by correspondingly few engorged females. The itchiness abated soon after all engorged ticks had detached from the calves and this conforms to results from primary experiments. The guinea pigs on the other hand continued to attack the infested areas for much longer after

detachment of all engorged ticks and thus inflicted severe traumatization. The response in calves exposed to larvae and nymphs demonstrated that primary exposure to the agent(s) of R. appendiculatus can provoke pruritis, though this reaction was not specifically observed during the primary experiments.

10.2.3. The integument, in contrast to other systems, sustained quite consistent responses which were marked and durable. These results confirm the observations obtained during the primary experiments. However, the calves, as opposed to the guinea pigs, developed much severer reactions which culminated into markedly distinct after effects. The distinction could be attributed to relative difference in areas exposed to infestation. The ears of calves had much smaller surface areas than the trunks of guinea pigs. Nevertheless, moderately high densities of nymphal infestation provoked identical severe reactions on the back of the guinea pigs which survived. The inflicted wounds and resultant inflammation produced an almost continuous scabby surface which was denuded of hair in several places. The calves showed bite wounds, as the primary lesion, accompanied by inflammation (redness, heat, pain and swelling) and severe exudative reaction. The surface of the affected ears became thickly encrusted (necrotic epidermis, blood, exudate and dead ticks) and fissured but the encrust remained firmly anchored to underlying tissue for some time. Premature manual disturbance or even vigorous

shaking of ears caused bleeding from the fissures.

Another reaction, unnoticed during the primary experiments, was detected within the infested and contiguous areas of the skin in three calves. The cutaneous lesions which were elevated, firm and nodular (papule) measured up to 1.5cm diameter and occurred round parotid, occipital and anterior cervical regions. With time they became exudative in the centre and as the exudate accumulated the hair over the lesion got matted. Subsequently the formed scab was surrounded by ^a/necrotic core which left a shallow crater of granular surface or lightly pigmented scar on removal. The erect hair tufts over these lesions could be spotted from a distance, but they were detected in only a few animals. However, it is possible they were missed previously as the literature describes identical lesions in relation to repeated exposures. Apparently at the time they were observed pressure did not reveal tenderness in the affected areas. The lesion was connected with larval and nymphal but not adult infestations. Microscopic examination of scrapings from the lesion revealed no parasitic or bacteriological organisms. The active reactions in the infested areas, on the other hand, were followed by marked scarring, extensive alopecia (depilation) and pityriasis and where very severe dermatitis developed the conchal cartilage became distorted. Thus the pinna(e) showed wrinkled edges, curled tip or the necrotic part felt like a dry hide. These deformities could be attributed to necrosis and scar formation in the tissues of the pinna. Healing was generally rapid

under the scab. Nonetheless, complete hair regrowth took more than a month in severely affected ears. In some calves practically the entire outer surface of the pinna was denuded of hair. The ears in guinea pigs were used briefly and then abandoned for technical reasons, e.g. device to shield feeding ticks. Subsequently the rest of the exposures were applied to the trunks. Besides the isolated severe reaction associated with nymphal exposure mentioned above, the bite wounds were generally accompanied by minimal exudation. There was sparse loss of hair and light flaky crusts from the exudate formed in the infested areas. However, detachment was followed by appreciable protracted bleeding and persistent red spots in the infested areas. The deranged coagulation process and the red spots indicate persistence of the effects of the agent(s) after detachment. Healing in guinea pigs developed rather slowly and was further delayed by the common attack on the exposed sites after the ruff restraint was removed. The severely traumatized areas had to be dressed to reduce secondary infection and further injury. Preparations containing antimicrobial, astringent and antipruritic ingredients were used (Calmine lotion + sulphanilamide powder). These dressings effected rapid responses and facilitated scabbing over raw surfaces.

In contrast, the salivary gland extract (SGE) inoculated intradermally/subcutaneously (i.d./s.c.) in

guinea pigs caused only cutaneous nodular reactions (< 1.0cm) at the site. The inoculations were made over the belly or flank. With time the epidermis over the nodules became necrotic and peeled off slowly but the nodules persisted for an indefinite time before resolving. The control sites inoculated with phosphate buffered saline or physiological saline solution left no impression, thus the nodular reaction was caused by the agent(s) in the SGE.

The clinical reactions induced during the investigations on the hypothesis were commonly observed in the first infestations of the primary experiments. However, some outstanding differences emerged in severity and range of lesions induced and the guinea pigs presented signs previously not associated with primary exposure to the agent(s). The noted reactions were severe dermatitis accompanied by bleeding from fissures in the encrusted epidermis, extensive alopecia, exudative papular eruptions and distortion of the pinna. The guinea pigs responded somewhat differently in relation to persistence of red spots and protracted bleeding in the infested areas after detachment of all engorged ticks. The persistent bleeding from the bite wounds indicated some disturbance in the blood coagulation mechanism. There was impulsive grooming and the intense pruritis persisted much longer in the guinea pigs. Thus the cutaneous responses to first exposure of the agent(s) confirmed the observations obtained during the primary experiments and further

demonstrated reactions previously undetected. The response in guinea pigs also demonstrates that effects of the agent(s) can persist in the host tissue after the exposure has ceased.

10.2.4. Little can be commented on the changes in the blood composition since only few random samples for haematology were collected. The reasons for the limited samples which include time and cost constraints have been stated (C.10.1). However, blood taken from the calves showed no significant alterations, but the guinea pigs showed watery blood in some cases and smears revealed high frequency of eosinophils and reticulocytes. The frequency of eosinophils demonstrated response to parasitism and the reticulocytosis associated with watery blood indicated critical anaemia. Nonetheless, appearance of reticulocytes alone in the species is not regarded as absolute as the cells can be commonly seen under normal physiological conditions (Schalm,^{et al.} 1975). It is noteworthy that high numbers of circulating eosinophils were not detected in calves during the primary experiments. This response could partly be attributed to sensitivity of the guinea pig as few workers have reported absolute eosinophilia in cattle infested with ticks (van Rensburg, 1959; Hewetson, 1971; O'Kelly, et al., 1971). Thus the response in guinea pigs demonstrated that the effects of the agent(s) mediated through first exposure to high density of R. appendiculatus instars causes serious changes in blood composition.

The immune responses deduced from reactions manifested in primary experiments were verified by the cutaneous Arthus reaction test (C.A.). The salivary gland extract (SGE) and feeding larvae were used as the antigens for the challenge. The test was applied to both types of hosts sensitized by ticks and SGE. The controls for the test were ~~a~~ affected by SGE administration in a proportion of the hosts, and phosphate buffered saline blanks (PBS) after sensitization. The results were negative. The calves were tested with SGE and larvae and the guinea pigs received only SGE. All guinea pigs and calves tested responded strongly to salivary gland extract. The intensity of reactions which reached their maximum in six to twenty four hours was rated mild to intense (++ to ++++). Overall the reactions were of immediate type though some cases developed rather slowly (evaluation based on erythema, oedema and skin fold changes: mild, ++; moderate, +++; intense, ++++.) The calves showed some variation whereby the maximum intensity developed in one to eight hours of testing and the reactions were rated moderate to intense. The response demonstrated a positive Arthus-type reaction. However, further tests with larvae in two calves given SGE on the previous day produced additional significant changes at the sites inoculated. The reaction to SGE had subsided and low grade degenerative changes were developing. The superimposed larvae provoked, within three to four hours, exaggerated erythema and massive exudation of serum at the SGE sites. The

larvae were applied to ears and the SGE to skin of the flank. The original reaction zones which measured about 2.5cm diameter became distinctly delineated. The plaques formed appeared red-brown indicative of an organising haemorrhagic lesion and progressed to necrosis. Necrosis was complete in two days and the colour turned brown against a non-pigmented background. The severity of the response indicated a high antibody level to tick agent(s) (Roitt, 1974) and represented an advanced degree of an Arthus-type reaction (Herbert, 1970). Herbert's (1970) description of the clinical signs associated with this degree of response included intense congestion, haemorrhage, oedema, necrosis, exudation and slow healing lesion of the reaction site. The signs are caused by circulatory disturbances. The responses observed in my calves were identical to the above signs. However, on the basis of the studies of Roitt (1974), the pathogenesis of the local reaction can be described as follows.

The union of soluble antigens and antibodies within the body may give rise to an acute inflammatory reaction, e.g. an erythema and oedema, which reaches a peak at 3 to 8 hours then usually resolves. The intradermally injected antigen precipitates with antibody, often within the venules, and the complex binds the complement. The fixation of complement leads to generation of anaphylatoxins and these cause histamine release with vascular permeability changes. The local intravascular complexes will cause platelet

aggregation and vasoactive amine release. The chemotactic factors also produced will lead to an influx of polymorphonuclear leucocytes which begin the phagocytosis of the immune complexes; this in turn results in the release from polymorph granules of proteolytic enzymes, kinin-forming enzymes and polycationic proteins which increase vascular permeability through both mastocytolytic and histamine-independent mechanisms. These will damage local tissues and intensify the inflammatory responses. Under appropriate conditions the aggregation of platelets may provide yet a further source of vasoactive amines and may also form microthrombi which can lead to local ischaemia. Thus the development of necrotic changes observed in my calves could be attributed to thrombosis and massive infiltration of polymorphs in the local tissues. Another striking feature was the marked tenderness at the tested sites which on palpation incited kicking. Concurrently, generalised intense erythema appeared all over the body in pigmented areas. The challenged ears also developed intense erythema, oedema, exudation, and a heavy crop of hypersensitivity blebs. The animals evinced irritability and pain over the ears and became restless. The feeding of the larvae was drastically inhibited. The necrotised

plaques at the inoculated (SGE) sites eventually sloughed leaving shallow crater scars. Thus the cutaneous reactions to challenge test with tick antigen demonstrated that first exposure to the agent(s) of R. appendiculatus induced strong precipitating antibodies conforming to standard description of an Arthus-type reaction.

The identical reaction to challenge with larvae in calves demonstrated that primary exposure to the agent(s) induced effects capable of inhibiting the successful feeding of ticks during subsequent infestations. The inhibition of tick performance thus supported the observations made during primary experiments, whereby the larvae were virtually annihilated within a day through hypersensitivity reactions. It was further shown that the immune responses were measurable as early as ten days following primary exposure. The intensified circulatory reactions (erythema, oedema, exudation) provoked by challenging the sensitized hosts confirms the responses attributable to^{an} immediate hypersensitivity state, though guinea pigs also showed a delayed type response. Thus the identical reactions observed during primary experiments can be attributed to immune responses to secretions of R. appendiculatus.

10.2.5. Exposure to feeding R. appendiculatus ticks, but not salivary gland extract (SGE), caused eight deaths in guinea pigs. The deaths occurred during and after exposure. Calves withstood the agent(s) of the feeding ticks and this was predictable from the overall mild systemic disturbance. The severe systemic disturbances, e.g. pyrexia, anaemia due to exsanguination, anorexia and

rapid prostration could have contributed significantly to the deaths. Thus exsanguination and general intoxication were incriminated for the death and supporting evidence was revealed on autopsy. The succumbed guinea pigs demonstrated that intensive primary exposure to effects of the agent(s) of R. appendiculatus can cause rapid and critical disturbance with fatal results. The autopsy changes representing the range of lesions produced in individual guinea pigs were as follows.

Carcass condition was generally good which conformed to the brief course of reaction. There was ample adipose tissue distribution. The subcutis, including the pelt, revealed light to extensive haemorrhages e.g. petechiae, ecchymoses and splashes. The haemorrhages on the pelt corresponded to the attachment sites which were distinctly marked on the top of the skin. The blood vessels were engorged and blood flowed freely (from them) even in carcasses autopsied more than fifteen hours after death. The free flow of blood indicated that coagulation mechanism was inhibited and corroborated the clinical persistent bleeding from detachment wounds. Otherwise the anaemic cases revealed pallor and dropsical degeneration. Both superficial and deep lymph nodes were enlarged slightly, congested or haemorrhagic, and oedematous.

The peritoneal cavity showed ascites (one guinea pig), pallor or congestion of parietal and visceral serosa and engorged vessels on intestines. The spleen commonly presented^a/deep red colour and in some cases appeared light

brown. The livers were light to dark red-brown or congested with free blood flow from cut surface (three carcasses). Some cases revealed haemorrhagic spots and mottling. Kidneys were pale or congested, haemorrhagic and enlarged with free blood flow from cut surfaces. The adrenals and uterus were similarly affected. Stomachs were empty or partially filled and carried mucosal haemorrhages in some. Intestines were similarly affected but the empty gut trapped gas and in some the contents were haemorrhagic, however, washing out revealed more serious congestion and haemorrhages. The thoracic cavity showed mild hydrothorax. The hearts showed consistent changes including hydropericardium, prominent coronary vessels, light brown to congested myocardium, endocardial haemorrhages, fragile 'chicken fat' clots in the ventricles. The clot composition is characteristic of slow blood coagulation. Lungs bore dispersed areas of congestion, oedema and hepatization. The body muscles appeared pale/light brown to dark red (normal).

The autopsy changes corroborated the antemortem manifestations. Thus a number of clinical symptoms were made more meaningful on autopsy. The persistent bleeding was associated with uncoagulated blood and chicken fat clots in different guinea pigs which indicated the factor responsible was in the circulation. It could be suggested that the effect of the agent(s) caused anticoagulant activity. Diapedesis observed round bite wounds could also be attributed to circulatory agent(s) that deranged the

endothelium leading to haemorrhage and congestion in the internal organs. The renal changes partly explained the oligouria evinced in the guinea pigs. However, renal damage could further disrupt the biochemical equilibrium as a result of accumulated waste products, e.g. ammonia and other waste nitrogenous by-products which cannot be eliminated normally. The rather sudden prostration was unlikely to have resulted from such direct biochemical complications; thus effects preceding death could be attributed to general intoxication caused by circulating agent(s).

10.2.6. CONCLUSIONS

The primary exposure to the agent(s) of Rhipicephalus appendiculatus through tick feeding and salivary gland extract (SGE) inoculation induced distinct responses in both hosts which culminated in death of guinea pigs infested with ticks. The major responses included pyrexia and hypothermia (guinea pigs); papular and exudative dermatitis followed by alopecia (depilation) and deformation of pinnae; circulatory disturbances - anaemia, congestion, oedema and haemorrhages, especially in guinea pigs; anorexia and oligouria (guinea pigs); pruritis; hypersensitivity state revealed by cutaneous allergic test; rapid death in guinea pigs. The hypersensitivity state reactions were of the immediate type in both hosts though the guinea pig responses also suggested a delayed type. These changes were attributable to exsanguination

and intoxication phenomena. The autopsy findings corroborated the clinical signs manifested and the changes observed in tissue were predominantly related to circulatory system derangement. Guinea pigs treated prophylactically with antibiotics succumbed to effects of the agent(s) of R. appendiculatus and this further supported the toxæmia phenomenon. The results from these investigations corroborated the observations from the primary experiments, though guinea pigs presented extreme reactions which were not seen previously in infested calves. Therefore the responses of the calves and guinea pigs to the agent(s) of Rhipicephalus appendiculatus did not confirm the hypothesis that, 'the agent(s) cannot provoke clinical-pathological response on primary exposure'.

10.3. EXPERIMENT (VII.2): RESPONSES TO HYPOTHESIS (II)

The hypothesis stated, 'The agent(s) cannot produce a clinical-pathological effect that is passively transmissible by immune serum'. A number of workers have reported on transferrable effects from sensitized to susceptible hosts using immune serum or lymphoid cells (Riek, 1962; Trager, 1939a; Roberts & Kerr, 1976; Wikel & Allen, 1976a; Various host-tick systems were used, e.g. Dermacentor spp. + guinea pig; B. microplus + cattle, for production of immune sera which was

administered by different routes to the recipients. The passively transmissible effects were monitored by tick feeding or allergic tests-passive cutaneous anaphylaxis (PCA). The monitored results varied and in some cases inconclusive responses were obtained, however, the PCA gave more consistent responses. The assumed development of immune responses to agent(s) of R. appendiculatus was demonstrated by the investigations of hypothesis (I) (C.10.2.4.).

Thus the investigations of the hypothesis (II) were implemented by intradermal (i.d.) administration of up to 1.0ml homologous immune serum twelve to forty eight hours before challenge with salivary gland extract (SGE) at the sensitized sites. The donors were exposed at least twice to tick infestation before bleeding. Controls for the passive cutaneous test for Arthus reaction included SGE challenge at the sites inoculated with non-immune serum and uninoculated; and phosphate buffered saline challenge at sites sensitized with immune serum. The reading of the tests commenced about thirty minutes after administration of SGE and the scoring followed the same features as stated in C.10.2.4.

10.3.1. The induced reactions at the sensitized sites were as follows. The calves gave a positive reaction which was of a mild to moderate intensity (++ to +++) and the reaction reached a peak in about one hour. The reaction subsided appreciably in six hours and by next day

the inoculated sites were just discernible. The most intense reactions appeared at sites sensitised with highest doses of serum (approached 1.0ml) and the assessments revealed an increase of three to four times in the erythema/oedema zone and one and a half to two and a half times in the thickness of the skin fold. The guinea pigs in contrast gave slow response which showed just mild erythema and marginal increase in the zone by two hours. However, by sixth hour marked reactions became evident, i.e. the intensity increased to over two times in erythema/oedema zone and one and a half times in the skin fold. The resultant lesion at the reaction site was still apparent up to four days later. It is worth noting that one guinea pig given heterologous (bovine) immune serum sustained adverse local reaction and that challenge with SGE the next day provoked a violent response which culminated in immediate systemic anaphylaxis. The guinea pig whilst in sternal recumbency evinced tachypnoea, tremors in the hind limbs and eyelids, cyanosis over ears, involuntary defaecation and by ^{the}fourth hour shifted into partial lateral recumbency. No resuscitative treatment was given but recovery signs commenced about the sixth hour. By next day the test site was markedly regressed. Conversely the control sites, in both hosts, showed no significant reaction (C.10.3).

10.3.2. Conclusions

Passive sensitization of susceptible hosts with immune sera from subjects exposed to the agent(s) of Rhipicephalus appendiculatus induced an immediate allergic reaction (PCA) demonstrated on intradermal challenge with salivary gland extract (SGE). The responses demonstrated corroborated responses from the different tick-host systems reported by previous workers. The positive reaction demonstrated the specificity between the immune sera and the agent(s) in the SGE of R. appendiculatus. Therefore effects induced by exposure to the agent(s) were transmitted to susceptible hosts by immune serum. Thus the investigations did not confirm the hypothesis, 'the agent(s) cannot produce a clinical-pathological affect that is passively transmissible by immune serum'.

10.4. EXPERIMENT (VII.3): RESPONSES TO HYPOTHESIS (III).

Previous workers demonstrated elevated blood histamine levels (Riek, 1955) and ^{the} influence of antihistamines on the host-tick responses (Tatchell & Bennett, 1969) in cattle repeatedly exposed to Boophilus microplus. High histamine levels were associated with immune responses in resistant cattle and the histamine was closely connected with mediation of clinical manifestations. Such studies have not been reported in cattle repeatedly exposed to Rhipicephalus appendiculatus. The relevance

of this statement is that varied responses have been produced under different cattle breed - B. microplus systems (Tatchell & Bennett 1969). However, the primary experiments in the present study revealed significant manifestations, especially in the circulatory system, which could be attributed to histamine effects. Thus the hypothesis, 'The agent(s) cannot produce a reaction of a type that can be blocked by antihistamines', was adopted to investigate whether the antihistaminic effect demonstrated in cattle - B. microplus interaction(s) could not influence the cattle - Rhipicephalus appendiculatus system. The guinea pig - R. appendiculatus was simultaneously tested.

10.4.1. The investigations involved administration of antihistamines to hosts actively and passively sensitized. The inclusion of passively sensitized hosts was a further test on the responses sought in hypothesis (II) (C.10.3.). The active sensitization to effects of the agent(s) was through tick feeding and the passive sensitization followed the same procedures described for hypothesis (II). The antihistamines used were tripeleennamine hydrochloride (2%) and promethazine hydrochloride (2% & 5%) solutions and were administered at varied dosage rates commencing just before or simultaneously with the challenge. The challenge agents included feeding ticks and inoculation of salivary gland extract (SGE). The controls to the trials were tested before or after the antihistamine administration to confirm^{that} the responses produced were mediated by the drug(s). The evaluation of the

response(s) was based on the clinical manifestations, tick performance and (passive) cutaneous Arthus reaction.

Thus the results are described under two sections, viz: responses of hosts exposed actively and passively to effects of the agent(s). Results for the influence of each antihistamine are presented separately.

10.4.2. Responses of hosts exposed passively to effects of the agent(s).

Only tripelennamine hydrochloride was administered to one calf and one guinea pig and both had not been previously exposed to the effects of the agent(s). The indicator for the influence of the antihistamine was the passive cutaneous Arthus reaction (PCA). The calf received only a single intramuscular (i.m.) dose of tripelennamine half an hour before i.d. challenge with salivary gland extract (SGE) and the reading commenced twenty minutes later. The initial inoculation plaque of SGE measured 1.2cm diameter and subsequent readings for over one hour revealed only mild erythema which was fading fast. However, the appreciable change was little elevation of the centre of the plaque skin. By next day the plaque (zone) was developing into palpable cutaneous nodule. This could be attributed to persistence of complexes from agent(s) and immune serum which were insensitive to the antihistamine. A control challenge a day later (without antihistamine) elicited slight increase in plaque zone, intense erythema and elevated surface (oedema). However, the intensity of reaction was less marked than that produced in

hypothesis (II). This could have been related to the concentration of serum inoculated and possible residual antihistaminic effect. Thus the trial demonstrated tripelennamine suppressed the PCA reaction. Similarly, the guinea pig treated one and a half hours before challenge and observed for several hours developed no significant reaction at the site. Further trials without tripelennamine administration was not done due to limited supply of SGE.

Therefore tripelennamine hydrochloride administered systemically inhibited the clinical signs of the passive cutaneous arthus reaction in both hosts.

10.4.3. Responses of hosts actively exposed to the effects of the agent(s).

Both tripelennamine and promethazine antihistamines were administered in the calves and guinea pigs. Previous exposure to the agent(s) through repeated tick infestations caused severe responses in calves. The challenge agent(s) were the SGE inocula and feeding immature instars. Antihistamines were administered before and during the challenge. Tripelennamine hydrochloride was tried in two calves. One calf (A) received a single injection and was challenged twice with SGE at one and four hours after administration of the antihistamine. The first challenge produced an initial plaque 1.5cm diameter which increased to 1.8cm at the fourth hour and was just palpable through the skin thickness. The second challenge at the

fourth hour produced an initial plaque 1.5cm which increased to 3.2cm diameter in another four hours. The plaque was much elevated at the centre and tender but by next day regressed to 1.2cm and was not tender. In contrast, the second calf (B) received multiple doses of promethazine commencing with application of larvae and continued five hourly in the day on the first day and twice daily thereafter. The frequent dosage on the first day was dictated by the response in calf (A) indicative of rapid decline in the therapeutic levels. The larvae settled quickly and in variance with the previous infestations no immediate severe epidermal reaction harmful to the ticks developed. However, reactions of relatively mild nature started on the second day post infestation (dpi). The changes observed first were increased warmth over ears, detachment of some larvae, scanty blebs (pustule type), intense itchiness and restlessness, early signs of larval engorgement. On 3dpi the calf appeared more restful and the larvae were definitely engorged and other signs were slightly subsided. On 4dpi the outside of the ear developed a thick necrotic epidermis where larvae clustered and the sites were moist from exudation. The inside of the ear became sweaty and red and the itchiness was still marked (calf stood still when the ear was gently scratched). The engorged larvae detached (a sample of prematurely detached larvae stored at room temperature lived more than twelve days but did not develop to moulting). Through 5/6 dpi the pinna remained raw

but on 7 dpi was drying and scabs formed were lifting. On 8 dpi the pinna was virtually healed. However, a further challenge eight days later without antihistamine therapy provoked a rapid and markedly severe reaction that killed practically all larvae applied. The larvae were engulfed by the excessive exudation. The reaction developed within six hours of application of the larvae. The responses demonstrated that tripelennamine exerted significant blockade effect on the reactions provoked by the agent(s) of R. appendiculatus. Thus the inhibition exerted promoted successful feeding of the larvae and accelerated resolution of the lesions produced. The effective action of the antihistamine clearly demonstrates that histamine plays a dominant role in the mediation of clinical-pathological manifestations associated with immune responses.

10.4.4. THE PROMETHAZINE HYDROCHORIDE trials involved two calves and two guinea pigs which had been previously exposed to the agent(s) through repeated infestations of Rhipicephalus appendiculatus. Challenge during antihistamine administration was effected by salivary gland extract (SGE) (subgroup C) and feeding nymphs (subgroup D). Previous tick infestations provoked severe reactions, especially in the calves. Subgroup (C) challenged with SGE received single doses of promethazine. The promethazine was administered to calf (C) one hour before SGE challenge and the readings taken at one hour and a

half, six hours and next day. The initial plaque at the challenge site measured 1.8cm diameter and at the first reading showed 2.0cm diameter, marked erythema and marginal increase in skin fold. The second reading six hours after challenge revealed no apparent change except slight increase in the fold and by next day the plaque was barely discernible. The control test a day before administration of antihistamine provoked intense erythema/oedema and moderate increase in skin fold. The measurements of the plaque were, the zone increased from 1.8 to 4.5cm diameter and skin fold increased two and a half times and by next day the plaque was distinct and indurated. The guinea pig (C) was similarly treated and the first reading, one and a half hours after SGE administration, revealed minimal erythema or skin fold changes and by six hours the plaque was just discernible. A further confirmatory challenge ten hours later, without additional promethazine dose, produced an identical reaction and by next day the plaque had reduced to ^a/small red nodule. The similarity between the two reactions could be attributed to persistence of promethazine in the tissues and this differed from the activity of tripelennamine.

10.4.5. The variation in trials on promethazine hydrochloride effects was made by using nymphs instead of salivary gland extract as the challenge agent(s) and this involved one calf (D) and one guinea pig (D). Multiple doses of promethazine were administered commencing

at the application of the nymphs and thereafter at different intervals. However, repeated doses on the first day provoked toxic side effects described in C.2.16.8. Thus the administration was temporarily suspended until the signs of toxicity had disappeared but the dosage rate was subsequently reduced. Despite the adverse side effects the calf did not develop the severe hypersensitivity reaction that occurred in the preceding exposure without antihistamine administration. The clinical manifestations were minimal and the significant signs were mild erythema, few blebs of pustule type and mild pruritis by 4 dpi. The ears ultimately developed necrotic and haemorrhagic lesions around detachment wounds. The guinea pig (D) was rapidly overwhelmed by the massive exposure to nymphs of R. appendiculatus despite administration of promethazine for two days. The quick settling of the nymphs provoked ^{an} immediate pyrexia reaction that suddenly regressed to hypothermic levels next day. A few hours of nymphal feeding led to generalised erythema of extreme intensity especially along the edges of eyelids, nostrils and cloacal lips which were prominently pencilled. On the second day the guinea pig became extremely weak, anorexic and finally prostrate culminating in death on the third day.

The ticks settled within six hours of application on both hosts and fed normally. However, a small proportion died in situ on the calf where complete engorgement and detachment occurred 4 to 6 dpi. Engorgement was not

completed on the guinea pig which succumbed earlier. Promethazine hydrochloride administration promoted significant degree of successful performance of the nymphs applied.

Thus administration of promethazine effectively suppressed the severity of the induced responses to the agent(s) and facilitated the successful performance of nymphal Rhipicephalus appendiculatus in contra-distinction to similar exposures without antihistamines. These findings corroborated the responses demonstrated by Tatchell & Bennett (1969) using a bovine-Boophilus microplus system, though long administration of the antihistamines in their trials subsequently reversed the success of the tick performance because of boosted host resistance. Thus the reversed tick performance indicates the influence of the antihistamines is of a temporary nature. It can further be deduced that the antihistamine (s) cannot effectively arrest reactions mediated by intensive exposure to the agent(s) of Rhipicephalus appendiculatus. This deduction is illustrated by the guinea pig which succumbed to nymphal feeding whilst under promethazine administration.

10.4.6. The autopsy on the succumbed guinea pig (D) revealed the following pathological changes. The carcass condition as expected was good but the top side of the skin was extremely congested and the attachment areas were haemorrhagic. The subcutis including the pelt showed diffuse deep congestion and markedly prominent

vessels which mapped the entire pelt. The muscles were equally congested. The majority of inspected lymph nodes were haemorrhagic and slightly enlarged. The heart showed congestion and haemorrhage on the epicardium and prominently engorged coronary vessels. Lungs were congested and carried areas of hepatization. The peritoneal surface and the abdominal organs were similarly affected. The entire autopsy picture corroborated the clinical manifestations which represented an acute septicaemic reaction. The basic disturbance was associated with the cardiovascular system which broke down with massive haemorrhage in the whole body. In view of the fact that the clinical signs before death did not reveal convincing systemic hypersensitivity, the death could be attributed to an intoxication. The intoxication could have been caused directly by the injected agent(s) and/or indirectly through antigen-antibody complexes described by Roitt (1974). If this was true then the antihistamine administration would not have effectively arrested events that culminated in death.

10.4.7. Conclusion

The investigation on the hypothesis, 'the agent(s) cannot produce a reaction of a type that can be blocked by antihistamines', produced findings that corroborated previous studies made on the influence of antihistamines upon Boophilus microplus - cattle responses. The succumbing of the guinea pigs under promethazine administration further emphasised the limitation in the efficacy of the drugs during boosted host resistance to tick effects

and overwhelming exposure to the agent(s). There is a possibility, however, that the dominant specific effects of the agent(s) are unrelated to histamine and consequently administration of adequate doses of antihistamines would be ineffective. The evidence derived from the reaction of the guinea pig (D) would strongly support direct intoxication of the body tissues through injection of the agent(s) by Rhipicephalus appendiculatus. However, the mechanism of this intoxication cannot be explained. Nonetheless, ^{the}role of histamine cannot be completely eliminated as it has been consistently shown that previously sensitized hosts develop high levels of blood histamine on challenge (Riek, 1955; Code, 1939). This would be relevant to guinea pig (D) as it had been previously sensitized. Despite this shortcoming tripelennamine hydrochloride and promethazine hydrochloride significantly inhibited ^{clinical}the/ responses to the agent(s) mediated through active and passive sensitization of calves and guinea pigs. Simultaneously their administration facilitated the successful performance of immature instars of Rhipicephalus appendiculatus which had failed without treatment. Promethazine gave more durable therapeutic levels than tripelennamine. The effectiveness of the antihistamines administered showed that some of the by-products of the interaction between the agent(s) and tissues of previously sensitized hosts included histamine and/or other active amines responsible for mediation of the clinical-pathological manifestations. Thus appraisal of the responses revealed by the investigations on the

influence of the antihistamines on the interaction between cattle (guinea pig) and the agent(s) of Rhipicephalus appendiculatus showed the hypothesis was not wholly false.

10.5. EXPERIMENT (VII.4): RESPONSES TO HYPOTHESIS (IV)

The hypothesis stated, 'There is no difference between the effects induced by primary and repeated exposures to the agent(s)'. The primary experiments revealed that additional heavy infestations of Rhipicephalus appendiculatus instars provoked a more serious reaction in the exposed calves. The tick performance was similarly affected, especially the attachment rates. Rejection was mediated through visible hypersensitivity reactions ^{clinically} (blebs), and/inapparent responses which affected larvae mostly. These adverse host reactions further caused death during and after feeding and impaired the reproductive capacity of engorged females. However, some responses constantly accompanied both primary and repeated exposures, and such features included lymphadenopathy, erythema, pruritis, pyrexia. Thus the only means of distinguishing responses to primary and repeated exposure would be by comparing the severity and pattern of development. In this case the comparison would show such variations illustrated as follows. The erythema of moderate degree tended to be confined round the attachment sites during primary infestation whereas during repeated

exposures it became generalised and much more intense. It could be suggested that the reaction to primary exposure depended predominantly on ^{the} direct effects of the agent(s), and that the reaction to repeated exposure was mediated by concurrent activity of direct effects of agent(s) and of the immune response. Thus the intensity of the response to primary exposure would equally depend on the quantity and diffusion rate of the injected agent(s). The assessment of the intensity of reaction to repeated exposure would be more difficult as it would also depend on the degree of host resistance which varies a lot in different hosts. Similarly the pyrexia on first exposure showed one major peak coinciding ^{the} with/engorgement period of the applied instar, whilst during repeated exposure two peaks of identical magnitude to the primary response appeared. Conversely, there were manifestations that occurred only on repeated exposure, of which the clinical hypersensitivity reaction is the best example. It should, however, be stated that an inapparent hypersensitivity state develops during primary exposure as demonstrated in hypothesis (I) (C.10. 2.4.). Pruritis assumes an intermediate position whereby in the primary exposures it was generally insignificant and brief whereas in the repeated exposures very intense and durable pruritic reactions developed. This distinction would be relevant in choosing cattle hosts for tick feeding studies where susceptible ones cannot be readily identified. It is possible ^{that} cattle exposed repeatedly to R. appendiculatus

will manifest boosted resistance to subsequent infestations as observed in the rabbits (Bailey, 1960, Branagan, 1974). Extreme responses, such as rapid death through exsanguination and intoxication, are possible during massive primary and repeated infestations. However, some differences are liable to emerge at autopsy depending on the density and duration of the exposures whereby the repeated infestations produce changes relevant to protracted injury, e.g. wasted tissues. Tangible differences have been revealed in other host-tick systems, e.g. cattle - B. microplus; guinea pig - Dermacentor andersoni, assessed histologically, clinically and by tick performance (Riek, 1962; Tatchell & Moorhouse, 1968; Schleger et al., 1976; Allen, 1973; Hewetson, 1971; Hewetson & Lewis, 1976). Thus the hypothesis was designed to seek whether there are no differences between responses to primary and repeated exposures to Rhipicephalus appendiculatus agent(s).

10.5.1. The investigations were thus based on two broad sub-groups, viz: the first comprised hosts exposed once to the agent(s) and the second comprised hosts exposed more than once to the agent(s) of the R. appendiculatus. The exposure to the agent(s) was effected by R. appendiculatus instars in calves and guinea pigs and salivary gland extract (SGE) in guinea pigs. The first subgroup was based on the findings from the investigations on hypothesis (I) (C.10.2.) and the second subgroup included the survivors from/first subgroup supplemented by a few new hosts making thirteen calves and nineteen guinea pigs.

Thus procedures involving the first subgroup will not be described hereafter. The second subgroup was exposed to ticks but in addition the use of SGE antigen during the testing for cutaneous hypersensitivity gave a supplementary stimulus. The exposures^{*} varied in density, frequency and instar between individual hosts. Rhipicephalus appendiculatus ticks were applied to ears of calves and trunks of guinea pigs, facilitated by calico sleeves. A proportion of hosts were prophylactically dosed with antibiotics (oxytetracycline hydrochloride injection & powder, and penicillin-dihydrostreptomycin suspension) to control possible secondary complications liable to mask responses to specific effects of the agent(s). Daily physical examination followed the same format as for the primary experiments. Some of the animals were tested for cutaneous Arthus reaction using salivary gland extract (SGE). Tick performance was monitored as before but occasionally their viability was tested in parallel using susceptible guinea pigs. This step was undertaken in anticipation of possible rejection from inapparent reactions. Indeed such rejection, reproduced twice, appeared within twenty four hours of application in one calf. The parallel exposure in two guinea pigs produced successful performance of the batch of larvae used and thus revealed the rejection was host mediated. Clinical-pathological samples were very limited for reasons stated before (C.10.1). Autopsies were prepared on the dead animals as for primary experiments

* See Table 10.4.1.

TABLE 10.4.i.

Experiment (VII.4): The density and frequency of secondary exposures to R. appendiculatus instars in calves and guinea pigs infested at 7 - 14 day-intervals.

Instar	EXPOSURES		HOSTS	
	Density*	Frequency	Calves	Guinea Pigs
Adults	220 - 600	3- 6 x	5	.
	50 - 250	1- 3 x	.	5
Nymphs	2500-5000	3-5x	4	.
	400 -4000	1-2x	.	9
Larvae	20000-50000	3-5x	4	.
	2000-20000	1x	.	5

* the numbers given are the ranges for ticks applied to individual hosts per exposure.

TABLES 10.4.

COMPARISON OF RESPONSES TO PRIMARY AND REPEATED EXPOSURES TO THE AGENT(S) OF RHIPICEPHALUS APPENDICULATUS

IN CALVES.

PARAMETER AFFECTED	E X P O S U R E S	
	PRIMARY	REPEATED
B/C	(+)	++ to +++
D/P	NAD	++++ depression, weakness & prostration
CVS:		
Erythema	++ localised	+++ generalised
Oedema	+ ears	++ ear etc.
Mucosae	Congestion - lachrymation	same & haemorrhage/discharges
Pyrexia	NAD/+	NAD/+
Blood	NAD	Anaemia
L/N	NAD/+ to ++	++ to NAD
GLT	NAD	++ (appetite)
Pruritis	NAD/+ brief	continuous +++, tender ear
Integument	Eczema, exudation, necrosis papules & transformation (++)	same & +++ & bleeding fissures, hypersensitivity, 'pox' scars.
CA	+ve immediate	Same
Death	-ve	+ve (3 calves)

B/C = body condition, D/P = demeanour/physique, CVS = cardiovascular system, GLT = digestive system, NAD = No abnormality detected, L/N = lymphadenopathy, CA = cutaneous Arthus reaction; very slight, + slight, ++ & +++ moderate, ++++ severe, +ve positive.

but no slaughter was undertaken for verifying changes in live animals. The results obtained from both types of exposures are presented in summarised Tables 10.4. and 10.5.

10.5.2. The significant responses to repeated exposure to the agent(s) of R. appendiculatus are described in more detail according to ^{the} systematic examination followed. Tick rejection was more marked and consistent and was principally associated with unsuccessful attachments and death in situ. Rejection occurred under both frank and in-apparent epidermal reaction. The influence of frank epidermal reaction was more marked in calves which developed severe clinical hypersensitivity reactions and epidermal necrosis. Repletion, viability and reproductive potential were also adversely affected, especially in females fed on guinea pigs. Such female ticks appeared small, discoloured, inactive and laid very small egg batches or none at all before death. The eggs hatched very poorly.

The host responses were markedly severe in some of the guinea pigs. Body condition declined severely in practically all guinea pigs and the loss was confounded by dehydration and inanition when terminally they did not eat or drink as desired. The calves, ^{the} in/absence of complications, were little affected initially but worsened with increased density and frequency of exposures. Demeanour and physique were also severely affected in guinea pigs which became depressed, drowsy and comatose terminally and prior to that developed inco-ordination and inertia. The calves

TABLE 10.5.

COMPARISON OF RESPONSES TO PRIMARY AND REPEATED EXPOSURES TO THE AGENT(S) OF RHIPICEPHALUS
APPENDICULATUS IN GUINEA PIGS

PARAMETER AFFECTED	PRIMARY	E	X	P	O	S	U	R	E	S	REPEATED
B/C	NAD to ++++										++++, confounded: dehydration etc.
D/P	+ to +++ depression, weakness pro- stration & coma.										
CVS:											
Erythema	++ & cyanosis or pallor										+++ generalised & cyanosis or pallor
Oedema	NAD										+++ droopy ears
Mucosae	+ congestion or pallor, discharges										+++ congestion or pallor; discharges
Pyrexia	NAD/ S/N/+ to +++										NAD/ S/N to ++
Blood smear	Watery blood: reticulocytes, eosinophilia										same
Respiratory	Dyspnoea under anaemia crisis										NAD
GLT	Capricious appetite, anorexia, con- stipation or diarrhoea										Anorexia, adipisia, constipation
Urinary	Oliguria terminally										Anuria terminally
Nervous	Impulsive pruritis but +++ traumatization.										same & paresis (20%)
Integument	+ Exudation, latent bleeding, red spots, necrosis & induration										+++ to ++++ erythema (generalised) necrosis, depilation, hypersensitivity.
CA	+ve immediate										+ve immediate
Death	+ve (33%)										+ve (43%)

B/C = body condition; D/P = demeanour/physique; CVS = cardiovascular system; GLT = digestive system;
 NAD = No abnormality detected; S/N = subnormal. CA = Cutaneous Arthus reaction.
 + slight; ++ & +++ moderate; ++++ severe, +ve positive.

were initially bright and active but later were depressed and generally weakened and fatal cases became prostrate terminally.

The cardiovascular system was similarly disturbed and manifested these changes. Erythema was singularly intense and generalised in guinea pigs which carried prominent red pencilling of the edges of natural openings (eyelids, nostrils, cloaca). The intense redness turned into cyanosis or pallor terminally. In calves erythema was constant, especially on the infested ears but became generalised in some. Oedema was localised and made ears droopy in guinea pigs (ears not commonly used though). The calves developed marked oedema especially during the early phase of tick feeding but it subsided before complete repletion of the applied ticks. Intermandibular oedema, which can also be attributed to lowered blood protein levels, appeared as body condition became poor. Pyrexia induced in guinea pigs showed moderate mean peak levels i.e. a rise of 0.7°C above critical normal temperature (arbitrary level 39°C) (uninfested means of rectal temperatures were 37.8°C a.m. and 38.7°C p.m.). A comparable response is illustrated in Fig. 10.1 for primary exposure. There was, however, some difference in the levels of primary and repeated exposures at the commencement of feeding. In both instances there was a peak coinciding with engorgement period. However, massive infestation precipitated hypothermia unpreceded by any rise in rectal temperature. Some of the guinea pigs sustained the hypothermia for two to three days before death. In

contrast only two calves exposed to adult R. appendiculatus developed a mild pyrexia as illustrated in Fig. 10.3. The mean peak levels were slightly lower than the primary (first) exposure reaction (Fig. 10.2) but the p.m. mean temperature rose earlier despite being virtually normal at engorgement time. It is interesting to observe that the pyrexia levels were much lower than during the primary experiments. The possible explanation would be the much smaller numbers of applied and engorged females during these secondary experiments. Similar to the guinea pigs, the calves which finally succumbed sustained a subnormal temperature for the last one to two days. The mucous membranes showed congestion and prominent vessels, especially on palpebral conjunctiva and sclera, excessive ocular and nasal discharges or pallor in the guinea pigs. Calves developed congestion and haemorrhages on the palpebral conjunctiva and lachrymation, especially on the infested side. Lymphatics sustained mild enlargement in the local drainage nodes. The degree of lymphadenopathy was lower than that observed in primary experiments, and this would be attributed to the much lighter density of adult R. appendiculatus applied. However, two of the calves did not show lymphadenopathy during the last exposures; this confirms the observation made during the primary experiments.

10.5.3. Digestive system disturbances in the guinea pigs were serious and culminated in anorexia/adipsia in

some and constipation. Constipation was followed by absolute suspension of defaecation during the terminal one or two days. Lack of faeces could be attributed to emptiness through inanition. The first exposure to the agent(s) produced an identical response in the guinea pigs (C.10.2.2). Calves generally maintained keen appetite, though feed intake declined under deteriorated clinical condition. Nonetheless, eating continued practically to the end as long as the feed was near the head. An unestablished enteritis complication developed in two of the calves that succumbed. The decline in condition was partly caused by the enteritis. Urinary function was adversely affected in guinea pigs which developed anuria for up to two days before death. This could be attributed to simple adipsia terminally, but kidney changes (congestion, enlargement and haemorrhage) as revealed at autopsy could possibly impair renal function. On the other hand no significant changes were detected in calves, except reflex urination when the ticks commenced feeding. The nervous disturbance observed in four guinea pigs was associated with loss of sensation in the hind limbs and impaired placing reflex (posture correction), convulsion and twitches terminally. The terminal convulsions and twitches could be attributed to depletion of energy metabolites since the animals had not fed for some time. However, this suggestion may not be tenable as such signs did not develop in the others which

succumbed after almost the same period of inanition. Pruritis could not be properly assessed in the guinea pigs because of impulsive irritability. However, the calves sustained severe pruritis throughout and shortly after tick feeding. The infested ear(s) became very tender during the pruritic phases. This response confirmed the observations made during the primary experiments.

10.5.4. The integument responses in the guinea pigs manifested generalised erythema, severe epidermal necrosis, loss of hair and hypersensitivity reaction (blebs of pustule type). The ears as described before became oedematous, markedly swollen and droopy and were darker than the rest of the skin. Removal of restraint devices after all engorged ticks had detached was followed by frantic biting at the infested areas, thus inflicting serious traumatization. The bitten areas were raw and became encrusted with serum/blood subsequently. The continued irritability could be associated with persistence of the effects of the agent(s) in tissues after all ticks have detached. Resolution of lesions in the absence of further injury took two to four weeks. Calves developed identical reactions but the hypersensitivity reactions were more intense and appeared fifteen to twenty hours and occasionally four to five days after infestation. The ears were thickly encrusted and often bled from the fissures in the firmly anchored encrustation.

Transformation involved temporary alopecia, persistent pityriasis and epidermal thickening. Cutaneous nodules (papules) of an exudative nature similar to the lesions described in hypothesis (I) (C.10.2.3) appeared over cheeks, behind ears, on the neck and anterior to the scapula spine. The nodular lesions were not recorded during the primary experiments, however, it is possible they were not identified and were missed. Healing followed the same pattern as in the primary experiments but left 'pox virus' scar marks in some areas.

10.5.5. The limited haematological samples taken revealed anaemic changes, such as, subnormal packed cell volumes, red blood cell counts, haemoglobin concentrations and poikilocytosis. The occasional preparations of scrapings from hypersensitivity blebs revealed increased eosinophil numbers and, to a lesser extent, other leucocytes. However, cytology preparations of the bleb fluid taken during the primary experiments revealed few healthy neutrophils and lymphocytes. Thus a representative picture of tissue changes requires proper histological preparation of the affected skin. The cutaneous Arthus reaction in the guinea pigs was identical to the reactions produced by the first exposure to the agent(s) (C.10.2.4). The resultant lesions at the i.d. test sites persisted for three to ten days before resolving or sloughing. The reaction in calves was as described for the control in hypothesis (III) (C.10.4.4.). It is noteworthy that one of the calves which succumbed

showed no obvious local or systemic clinical reactions to the fourth exposure of adult R. appendiculatus until it died on the seventh day of the same infestation. This non-responsiveness (or tolerance) followed shortly by death would indicate paralysis of the defence mechanism.

10.5.6. AUTOPSIES

Deaths occurred after one to three and three to six repeated exposures to the agent(s) in guinea pigs and calves respectively. Causes leading to death in calves were complicated by gastroenteritis of undetermined origin. The significant autopsy changes in both species were as follows.

Guinea Pigs. All exhibited marked wasting and severe septicaemic syndrome characterised by intense congestion and haemorrhages. Thus the lesions included extreme epidermal congestion and extensive congestion/haemorrhage and prominent engorged vessels on the pelt. Practically all internal organs and muscles showed identical changes and in some simulated an 'anthrax' septicaemia reaction. Dropsical changes in loose connective tissue and serous cavities were common. However, in cases where anaemic signs had developed the carcasses showed markedly subdued congestive changes. The septicaemic changes were very similar to those found in guinea pig (D) during the antihistaminic trial (C. 10.4.6.).

Basically the autopsy changes induced under the two

regimes of exposure to the agent(s) were the same, but the repeated exposures provoked much more intense reactions which developed faster such that death occurred before ticks were replete. The major features of contrast where repeated exposure produced worse reactions involved body condition and circulatory system. The circulatory disturbances were congestion (erythema of skin), oedema, haemorrhages and colour of the body muscles. However, pallor due to exsanguination was more common in the first exposure cases. This would be closely associated with the amount of blood sucked up to full engorgement. The ticks completed engorgement during most of the first exposures to the agent(s). Emptiness of the gastrointestinal tract was about the same but the inhibition of renal excretion was worse in repeated exposures. Another similarity was the delayed coagulation of blood associated with persistent bleeding (clinical) and free flow from cut surface of organs several hours after death. The appearance of worse reactions during repeated exposure would be attributed to the longer duration of exposure and immune responses. The immune responses are associated with release of (pharmacological) complexes responsible for mediation of physiopathological changes (Tatchell & Moorhouse, 1968). Thus repeated exposure to the agent(s) provoked a toxæmia that disrupted the endothelium leading to a fatal circulatory crisis before ticks were fully engorged. The reactions revealed at autopsy were faster in onset,

more intense and occurred in a higher proportion of guinea pigs from repeated exposure than from single first exposure regime. Overall death appeared earlier during repeated exposures. These autopsy changes cannot be compared with results of the primary experiments because no deaths occurred then in the calves.

Calves. On the other hand less drastic changes appeared in the three carcasses. The animals had lingered as condition deteriorated but the situation was confounded by the enteric disturbance. The death cannot be attributed to one dominant cause though certain features revealed were very similar to the changes induced in calves during the primary experiments. Thus the significant pathological changes observed were as follows. The carcass condition exhibited wasting and cutaneous lesions associated with clinical signs, e.g. encrustation, alopecia and scars on ears. Visible mucous membranes, especially conjunctivae on the infested side(s), showed oedema and haemorrhage but were generally pink. The subcutis showed evidence of dehydration or dropsy in different cases and wasted muscles which appeared very light red in colour. The lymphatics revealed slight to moderate enlargement and congestion/haemorrhage in regional drainage nodes (parotids). Spleens were 'wasted' in all and showed the following features: much reduced size, firm texture, light grey surface and cut surface dominated by white pulp. Livers appeared dark red/brown but in one case the edges were slightly enlarged and the parenchyma

was little mottled. The kidneys were congested and calyces/pelvis filled with gelatinous material. The abomasum exhibited oedema and congestion of the lamellae. The intestine/caecum manifested catarrhal inflammatory and haemorrhagic changes. The pleural cavity showed slight hydrothorax. The heart showed hydropericardium, prominent coronary vessels, epicardial petechiae and the flabby right ventricle. These changes would be associated with chronic wasting and toxæmia. Lungs were congested. Bone marrow consisted of red matrix in the epiphyses and diaphyses which indicated activity.

The autopsy changes of the three calves cannot be compared with any results from the first exposure to the agent(s) nor from the primary experiments because no corresponding deaths occurred in calves. Whereas enteritis complicated the situation, there were still certain features closely associated with chronic effects of exposures to the agent(s) and these conformed to the results of the primary experiments where calves succumbed to repeated infestations of adult R. appendiculatus. Such features included 'pox virus' scar marks, wasting and dropsical changes in connective tissue and muscle, conjunctival haemorrhage/oedema, sub-duced lymphadenopathy in regional drainage nodes, peculiar spleen 'hypoplasia', and other circulatory changes in various organs (heart & kidneys). The state of the bone marrow indicated stimulated activity as revealed by the few screening haematological estimations. Thus

the net syndromes were debility and circulatory disturbance which can be induced by chronic exposure to the effects of the agent(s) of R. appendiculatus. The findings from these autopsies do confirm the observations made on the corresponding exposure to R. appendiculatus infestations during the primary experiments. However, pallor of tissues in the calves was not a prominent feature in this hypothesis compared with the observation on primary experiments. This could be attributed to the lower density of adult Rhipicephalus appendiculatus applied.

10.5.7. Conclusions

Basically the pathogenesis of responses to both primary and repeated exposure to the agent(s) of Rhipicephalus appendiculatus was the same but the development of immune responses, e.g. hypersensitivity state, in the latter exposure regime significantly modified the pattern and severity of the signs and lesions. The repeated exposures surpassed the primary exposures in the severity of induced responses in the loss of body condition, cutaneous reactions, circulatory disruption, pruritis, clinical hypersensitivity state and rejection of ticks. Nonetheless, similarities were found in the integumentary changes (alopecia, deformities), pyrexia pattern (peak coinciding with engorgement) some functions of digestive system (guinea pigs - anorexia, constipation), anaemia, lymphadenopathy (calves) and mode of death (guinea pigs - hypothermia and intoxication

syndrome). It is noteworthy that the guinea pigs, as opposed to calves, reacted more severely and this could be attributed to susceptibility of the animals and to species differences. Therefore the interpretation of the results for both host species should make provision for these features. The existence of the revealed similarities and differences in the responses therefore renders the hypothesis, 'there is no difference between the effects induced by primary and repeated exposures to the agent(s)', not wholly true.

10.6. SUMMARY OF RESPONSES TO HYPOTHESES (I) TO (IV)

The investigations on the hypotheses which tested the observations from primary experiments revealed in general that the null-hypotheses were false. The findings confirmed many of the previous observations and further demonstrated a number of features which were not obvious during the primary experiments. The guinea pig model elucidated some phenomena which were rather inhibited in the infested calves during primary experiments. These were vividly manifested in the circulatory, digestive and urinary systems. The guinea pig further demonstrated the lethal effects of massive exposure to Rhipicephalus appendiculatus which could be closely associated with exsanguination and intoxication. Hypotheses (I) which stated, 'the agent(s) cannot provoke

clinical-pathological response on primary exposure', was shown to be false since the investigations revealed significant clinical-pathological manifestations that culminated in death of some guinea pigs. The salient reactions produced included pyrexia and hypothermia, anaemia, anorexia/adipsia, brief pruritis, development of immune responses (immediate cutaneous hypersensitivity) and death from massive infestation. Hypothesis (II) which stated, 'the agent(s) cannot produce a clinical-pathological effect that is passively transmissible by immune serum', was also found to be false. The investigations revealed that humoral immune effects of R. appendiculatus were passively transferrable to susceptible hosts through immune serum. The transferrable effects were revealed by passive cutaneous Arthus reaction test. Administration of antihistamines demonstrated that the drugs had an effective inhibitory effect on the clinical signs mediated by histamine released during the immune responses to the agent(s) of R. appendiculatus and facilitated successful performance of applied ticks where it did not occur before. However, promethazine hydrochloride could not prevent the rapid death of a sensitized guinea pig challenged with a massive infestation of R. appendiculatus. The anomaly showed that the syndromes associated with such infestations were not predominantly mediated by histamine. Thus the hypothesis (III), 'the agent(s) cannot produce a reaction of a type that can be blocked by antihistamines', was found partly true. The existence of similarities and

differences in host responses to single first and repeated exposures to the agent(s) of R. appendiculatus made the hypothesis (IV), 'there is no difference between the effects induced by primary and repeated exposure to the agent(s)', not wholly true. The similarities appeared in the integumentary changes, pyrexia pattern, anaemia, lymphadenopathy and mode of death. The differences were revealed in cutaneous reactions, clinical hypersensitivity state, rejection of ticks, disruption of cardiovascular function, and manifestation of pruritis.

CHAPTER 11.

DISCUSSION

11.1. GENERAL

Tick infestation causes severe effects which disturb the health of the host and can lead to death. Death follows massive and/or protracted moderate infestations of Rhipicephalus appendiculatus (Neitz, 1962). The role of these effects on the disturbed health can be direct or contributory in nature. Recognition of these specific tick effects was incidental to early investigations of the important animal diseases transmitted by ticks. Since then studies have been undertaken to gain more understanding of the complex host-parasite relationship involving different host-tick systems. The knowledge so far gained is being applied to establishing biological means to control tick populations and thereby reduce the ravages caused through feeding and transmission of disease. Workers in Australia are making intensive studies on the heritability of resistance to these serious tick effects, specially based on ^{the} cattle - Boophilus microplus system. However, knowledge of host-parasite interaction in ^{the} absence of infections is far from complete but the adverse effects inflicted in the host are fairly widely acknowledged. The appraisal of the responses to infestation involves observation on clinical, haematological, biochemical and pathological (histological, immunological) reactions. The information

obtained on each of these features varies markedly between different tick-host systems. Certain systems, such as Boophilus microplus - cattle in Australia, have been extensively explored whereas in Africa relatively little has been done on the important tick-domestic stock systems. Besides disease transmission the ticks themselves have assumed prominence in the economic farming in Africa where livestock, especially cattle, suffer serious injury accompanied by debility, poor growth and production and death. However, in most cases, these effects are incipient and overshadowed by the concurrent diseases. The losses thus incurred from these effects are devastating and seriously inflate the cost of production. The use of ixodocides and other managerial practices as control measures become less effective for peasant (small) farmers due to high overheads and development of resistance to ixodocides. The knowledge gained in other countries on biological control based on host responses can be adapted to African ticks. Neitz's (1962) review on tick-toxicosis in farm stock highlighted the devastation caused and this led to a recommendation for further studies on the problem. The suggested studies were to be based on clean animals submitted to massive infestations of clean adult R. appendiculatus in order to observe an uncomplicated clinical reaction. In addition studies on the histopathology of the infested animals was considered. the In/absence of transmissible infection and severe exsanguination the debility induced can appropriately be

attributed to toxic factors. Neitz describes the R. appendiculatus toxin as leucocytotropic, and thus it is not unreasonable to speculate that similar effects can be mediated in other tissues causing wasting and debility.

The present study, on a small scale, explored the effects of repeated infestations of R. appendiculatus on the health of cattle, represented by calves. A number of constraints, viz: cost, space and facilities, warranted use of guinea pigs during the secondary experiments. They served as a model to demonstrate and confirm the responses evinced by calves in the primary experiments. In undertaking the model approach it was recognised that marked differences do exist between various host-tick systems (Riek, 1956, 1962; Branagan, 1974; Yeoman, 1966). Nonetheless, the guinea pig offered several advantages, e.g. reduced cost, relatively small tick numbers required to provoke reaction, and high sensitivity to established tick feeding. Herbert (1970) states that the guinea pig is very sensitive to histamine in contrast with cattle which can withstand much higher levels before clinical manifestations appear. This characteristic was reflected in the secondary experiments when antihistamines failed to inhibit responses, e.g. intense erythema, in guinea pigs exposed to adult and nymphal instars of R. appendiculatus. However, this failure could be caused by different pharmacological principles, from the injected saliva or by-product of antigen-antibody reaction, which are not susceptible to antihistamines. The high sensitivity

made a tremendous contribution toward the very vivid picture induced in the circulatory system during both primary and repeated tick exposures. Calves showed fewer clinical-pathological changes which could be attributed to direct toxicosis. The significant adverse responses to first and repeated exposures to the three instars of R. appendiculatus showed varied patterns and severity between infestation regimes and between individual hosts. The reviewed literature on R. appendiculatus effects reports very little on clinical-pathological responses in cattle (Branagan, 1974; Bailey, 1960). However, Thomas & Neitz (1958) and van Rensburg (1959) in their planned studies on rhipicephaline toxicosis revealed a broadspectrum of responses in cattle but their results were complicated by intercurrent infections and other tick species which appeared concurrently. None of these studies systematically investigated the influence of individual or concurrent two/three instar infestations. Rhipicephalus appendiculatus, a three-host tick, is capable of parasitizing different hosts through its life cycle. The adult instar under field conditions tends to favour feeding on cattle (Yeoman, 1966). Moreover, under field conditions all three instars can infest one host concurrently. The concurrent three instar infestation, especially under high density, can cause dramatic responses. Thus Dr. M.P. Cunningham (personal communication) encountered unanticipated massive infestation of all three R. appendiculatus instars which was followed by rapid

death or moribund state in a number of steers. The episode occurred during Theileria parva transmission experiments in steers exposed to infected ticks in experimental paddocks. Rhipicephaline toxicosis was diagnosed as the immediate cause of death. On the other hand, Theiler (1921) demonstrated death from exsanguination caused by massive infestation of a horse and a heifer exposed to Boophilus decoloratus. Unfortunately his published findings do not indicate the progression of the physiopathological events leading to fatal anaemia.

Repeated infestation with high density of the three instars of R. appendiculatus applied individually or concurrently at weekly or fortnightly intervals induced varied effects. The infestation regimes of individual adult instar and the concurrent three instars of R. appendiculatus affected the calves most severely. This is predictable from the size of adults, feeding period to complete engorgement and the considerable amount of blood sucked, and much saliva injected during the maximum feeding phase. In the absence of a regular systematic physical examination the first sign noticeable will be loss of condition. However, systematic examination should reveal many other related and apparently unrelated disturbances. A critical appraisal of the observed syndromes would emphasize the inter-relationship between the varied manifestations induced. Thus, the calves whose health was deranged by repeated infestations of

appendiculatus presented the following manifestations.

11.2. Loss of body condition. This was progressive and involved deterioration of general body appearance. There was severe muscle wasting leading to prominence of bone points, hollowness of paralumbar fossae and eyes; deterioration in quality of the coat and general physique. The progressive deterioration was attributable to multiple factors which in most cases cannot be specifically explained. In order to gain more knowledge about the mechanism underlying such factors it would warrant undertaking more detailed and sophisticated physiological and biochemical studies. Deprivation was induced by the repetitive challenge whereby the animals fed less due to irritation. There was a frantic attempt to groom which became intensified during secondary exposures. The severe pruritis resulted from interaction between tick secretions and body tissues which released active pharmacological agents, e.g. histamine and similar compounds. The released agents mediate pain and irritation (Riek, 1962; Tatchell, 1969a; Kemp, 1978). The worry induced in my calves conforms to responses demonstrated by Koudstaal et al., (1978), Snowball (1956), Hewetson & Nolan (1968), Hewetson (1971) in cattle resistant to the cattle tick, Boophilus microplus. They observed that much time was spent grooming to obtain relief. Loss or failure in weight gain and stunting serve as measures of the status of body condition, but calves in my study were not weighed. However, visual assessment indicated severe loss in weight as the calves became

extremely emaciated. Francis (1960), O'Kelly & Spiers (1976), Williams et al., (1978), Seifert (1971) demonstrated significant losses in growth or weight gain in heavily infested cattle. The loss occurred even in animals fed adequate rations (O'Kelly & Seifert, 1970). Similarly Riek (1957b) and Seebeck et al., (1971) observed such animals sustained long convalescence after cessation of infestation despite adequate feeding. They suggested that the poor condition was an indication of prolonged toxic effects of salivary secretion, though such effects were thought transient in the absence of concomitant infections. These observations agree with my findings except for the duration of the convalescence where my calves showed rapid recovery. It is also noteworthy that the calves wasted progressively, despite continued feeding, virtually to the end. It cannot be true that poor feed conversion was the dominant cause of wasting, as the drastic reduction in feeding, especially on hay, occurred only during the terminal stages. It is interesting to note that even during terminal recumbency calves would eat when feeds were placed close to the head. The inconsistency was also found by Seebeck et al., (1971), and O'Kelly et al., (1971), during studies on specific and anorectic effects on ^{the} cattle response to infestation with Boophilus microplus. Lack of precision in the findings therefore left room for speculation on the role of specific tick effects on growth/weight gains. Nonetheless, their conclusions emphasize the subtle nature of the mechanism

involved and the multiplicity of factors leading to loss of condition and production. They observed that the failure to gain weight could be attributed mainly to reduced feed intake or to joint 'anorectic' and specific effects. The influence of the latter effects on the metabolism of the host was associated with toxins from salivary secretion (Riek, 1957b, O'Kelly et al., 1971). It was however, found that high nutritional status could have a profound ameliorating influence on the susceptibility to tick effects but several studies revealed that this could be eliminated by very heavy infestation (O'Kelly & Seifert, 1970; Gladney et al., 1973). Thus, (1970) O'Kelly & Seifert/stated that even adequate diet could not counterbalance the depression in growth rate and blood composition caused by heavy tick burdens. By analogy, poor ration would normally weaken the host and when fed for^a/prolonged period it leads to severe deterioration in condition (Utech et al., 1978a; O'Kelly & Seifert, 1970). In conformity with^{the}/above findings, the calves in the present study progressively lost condition and became extremely weakened despite adequate feeding. However, terminally the feed intake declined drastically because feeding became very sluggish. The sustained urge to feed shows^{that}/the calves did not lose appetite as would be the case in fevered animals. Poor digestion could be blamed for reduced conversion but the digestive tract functioned normally and the faeces passed were normal in quantity and consistency. However,

the guinea pigs differed in their response to both primary and repeated exposure, whereby moderate to absolute suspension of the digestive system function developed. Autopsy revealed ^apartially filled or empty gastro-intestinal tract which supports the deprivation cause of wasting. Since the onset of inanition (anorexia) was early in both exposures it would be reasonable to suggest that the depression of digestive system activity was induced by specific tick effects. Once the anorexia becomes established the body has to make up for the deficiency by deploying body tissue reserves in order to maintain the physiological equilibrium. The break down of tissues invariably causes further deterioration in the body condition as long as the host survives. The calves on the other hand did not suffer the drastic effects of anorexia. It can therefore be suggested that some factor(s) in the salivary secretion of R. appendiculatus induced debility independent of anorectic effects and that the fever, especially during adult infestations, was another major contributory factor. It can be further argued that metabolic dysfunction works at ^{the}cellular level although not much histological study has been done on vital body organs, e.g. liver. However, van Rensburg (1959) reported degenerative changes in liver cells - fatty degeneration, in steers which had been infested with ticks. The alteration in individual cells on a large scale can influence the function of the organs, thus upsetting the normal body metabolism. Nonetheless,

fat degeneration of the liver does not cause fall in the serum albumin levels. Deficiency of serum albumin would result in mobilisation of tissue sources in order to maintain equilibrium (Coles, 1974).

Tissue cells are bathed in extravascular serum substrate from which they derive their nourishment but disruption of the integrity of the vascular endothelium imposes a deficiency through leakage or haemorrhage. The induced accumulation of exudate/transudate in serous cavities or loose connective tissue deprives the body cells of the essential metabolites. Besides, the oxidative activity of the blood suffers because of impaired circulation and the resultant effects would accelerate cell necrosis. The guinea pigs portrayed a characteristic circulatory disruption which would lead to the deprivation changes described but significant wasting did not appear as death from intoxication set in rapidly. Another possible explanation for tissue wasting is dysfunction in organs which have been injured by specific or indirect tick effects, e.g. kidneys, whereby uncontrolled excretion of metabolites, which are normally conserved, develops. The autopsies revealed renal changes in dead calves and guinea pigs which could be suspect in regard to organ dysfunction. Thus some of the observations made in this study can form a basis for the induced loss in body condition.

11.2.1. It can further be observed that the skin responses due to direct and indirect tick effects as described

by many workers (Schleger et al., 1976; Tatchell & Moorhouse, 1968) could conceivably affect the internal organs providing sufficient tick secretions are injected into the host. The derangement in tissues can lead to severe haematological/biochemical changes which on their own can influence the body metabolism and ultimately cause deterioration in condition. These effects can be mediated through exsanguination that imposes a deficit in blood components, e.g. reduced erythrocytic indices, plasma proteins and electrolytes. The alterations in blood composition associated with tick infestation have been demonstrated by several workers; however, the relevance of some of them to loss of condition and production is not clear. O'Kelly et al., (1971), Seebeck et al., (1971), Williams et al., (1978) demonstrated ^{the} effects of ticks on ^{the} blood composition of cattle infested chronically with different ticks. One of the sequelae to deterioration of blood composition was a decline in body condition whereby animals failed to gain or lost weight. The causes could be associated with tick worry, direct loss of blood and altered biochemical changes all of which inhibited the normal physiological functions of the body. In corroboration the guinea pig model clearly illustrated the relationship between skin and internal organ changes as related to ^{the} circulatory system. Exsanguination ^{so} occurred/rapidly under massive exposure that the effects

of blood loss on body condition could not be properly assessed. It is possible that the drained components of blood were responsible for severe decline in physique (weakness) which normally remains sound under good body condition. The generalised circulatory disturbance indicates a high concentration of saliva in the blood stream and this pronounced manifestation could be related to the size of the guinea pigs as opposed to the calves. Thus the agents would have acted on the organ cells to produce wasting changes if the guinea pigs had survived longer. On the other hand, calves being much larger in size did not present a distinct relationship in the reaction between the integument and internal organs. However, significant decline in mean and individual haematological/biochemical estimations was induced in calves during the present study as illustrated in Figs. 6.6, 6.7, 6.14, 6.15. The mean values of successive estimations showed a regression against time which was significant in these parameters, red blood cell counts ($r = -0.81$ $P < 0.01 > 0.001$), haemoglobin concentrations ($r = -0.75$, $P < 0.01$), serum protein ($r = -0.63$, $P < 0.05$) and immunoglobulins ($r = -0.86$ $P < 0.01 > 0.001$). These calves were submitted to high density infestations of adult R. appendiculatus. However, certain individuals maintained normal levels despite the rapid loss of condition as opposed to others which concurrently showed loss of condition and marked regression in blood composition. This variation serves to emphasize the difference in response between individuals and breeds

observed by previous workers (Riek, 1962, van Rensburg, 1959). These critical changes in the blood composition accompanied by severe loss of condition were also found in the group exposed to the concurrent three-instar infestations of R. appendiculatus. Thus the critical decline in serum protein would cause marked loss of condition and disruption of the equilibrium of metabolites in circulation. The oedema that follows the hypo-proteinaemia impairs the normal body distribution of the metabolites. Besides, the living body has to restore the serum protein balance as will be described later. In contrast the infestations of the immature instars did not induce significant regression in blood composition despite the relatively high densities applied. However, they showed appreciable loss of condition which could be attributed to more subtle tick effects. It is noteworthy that in the field cattle are commonly parasitized by adult R. appendiculatus which are frequently accompanied by immature instars (Yeomann, 1966) and this would therefore increase the vulnerability of the animals even to moderate density of concurrent instar infestations. It is interesting to find that one calf among those severely affected survived the planned ten exposures and subsequently showed a rapid recovery in blood picture and body condition. The rapid recovery was attributable to much reduced numbers of successfully engorged females (Fig. 3.2) and maintenance of keen appetite. This response confirms the observations of Philip et al., (1935) that cessation of infestation (deticking) before animals became extremely affected was followed by rapid and complete recovery.

Guinea pigs, as described above, did not in general evince marked loss of condition before death but this change was appreciable in some which survived for some time. As opposed to calves, the effects were markedly associated with inanition and adipsia leaving the gastrointestinal tract virtually empty of ingesta. Persistence of this deprivation would invariably shift the status of the general metabolism leading to breakdown of body tissues (protein, fat) in order to maintain the equilibrium of energy supply and plasma protein. The plasma protein restoration would be more crucial when regression in the levels becomes significant. Thus delayed rectification of the imbalance would culminate in irreversible cachexia. Coles, (1974) describes the maintenance of the dynamic equilibrium between blood and tissue protein and states that where deficiency develops the blood levels are maintained at the expense of tissue losses. The suboptimal serum protein levels induced in the calves exposed to high density infestations of adult R. appendiculatus present the typical situation relevant to Coles's description. The irreversible loss of condition in the succumbed calves which sustained normal blood composition can be again attributed to more subtle tick effects associated with intoxication. On the other hand, lack of recovery in significantly regressed blood composition could be interpreted as severe inhibition of the mechanism described by Coles (1974) or complete exhaustion of mobilisable tissues. However, such a situation is

is difficult to conceive since these calves continued to feed and had clinically normal function of the digestive tract. It would appear that in general the body condition of calves, not showing pyrexia and/or significant alteration in blood composition, was influenced predominantly by specific tick effects.

It should be pointed out, however, that tissue breakdown for maintenance of the equilibrium of the metabolism does throw more burden on the excretory system as waste products accumulate faster than the body, especially in debilitated state, can cope with normally. Substances, such as nitrogen waste products and ammonia are toxic in high concentration and persistence of such levels inevitably paralyse normal physiological functions. It can further be suggested that the intoxication in organs from specific tick effects would aggravate the excretory burden. This situation could be associated more closely with the responses in guinea pigs where pyrexia, anorexia and adipsia developed. The cumulative effects of these subtle changes plus pyrexia, where it occurred, could have had a significantly synergistic role in the deterioration of condition in calves attributable to tick secretion intoxication. Furthermore reduced water intake or adipsia, as found in guinea pigs, does lead to dehydration. Dehydration, if unrectified in time, causes very adverse physiological disturbances followed by death. Thus it can be suggested that the loss of condition in calves was attributable to

several factors which could have acted singly or in conjunction with others. Nonetheless, the obtained evidence does not show that one single factor acted on its own.

The role of blood biochemical changes in the deterioration of body condition has been discussed above relative to total serum protein. It is noticeable from the estimation results for the various groups of calves infested with R. appendiculatus instars that only two groups (C.6.2 & C.9.2) sustained severe changes in the biochemical indices investigated. It is interesting to note that group (III) (C.6. exposed to high density adults) exhibited significant regression in the mean levels of the immunoglobulins ($r = -0.86$, $p < 0.01$ > 0.001) and this would support the suggestion made by Thomas & Neitz (1958) that rhipicephaline toxicosis suppressed the defensive mechanism of the reticulo-endothelial system. Thus animals showing such low levels in the immunoglobulins are bound to be more susceptible to infections, and should low levels persist it means inhibited production of antibodies. However, there was lack of significant differences in the mean values of immunoglobulins between the uninfested controls and infested group of calves during infestations (Table 4.19). This does indicate that the tick secretions did not provide strong immunogenic stimulus. In contrast the other groups exhibited no significant regression in the mean levels but analysis of variance

revealed some significant difference between the groups (Tables 4.18 to 4.20). Thus, on the whole the three indices investigated were stable and showed fair sensitivity to Rhipicephalus appendiculatus effects. These levels would not have caused the loss of body condition as described above. The indices have been described as stable relative to tick effects, this could be relevant to observations made by O'Kelly & Seifert (1970) who considered serum protein an unsuitable measure of tick effects on blood composition. They found an inverse relationship between albumin and globulins which dynamically maintains total serum protein levels. This is in agreement with results obtained by van Rensburg (1959) who found persistently high globulins whilst albumin declined under 'cachexic' condition. Thus O'Kelly & Seifert (1970) recommended the use of albumin as a more sensitive measure of tick effects. In this study only total serum protein estimations, which were optimal, were analysed as the albumin estimations were incomplete. Clinical oedema, which could have been attributed to hypoalbuminaemia, was not always easily detected; however, the autopsy on succumbed calves revealed dropsical changes. Thus the clinical diagnosis of hypoalbuminaemia in the emaciated calves was obscured by the serum protein estimations obtained. The reported hepatic changes in cattle infested heavily with R. appendiculatus (van Rensburg, 1959) would partly explain the effects of depressed serum albumin despite feeding adequate protein ration.* In view of these findings the use of albumin measure, as recommended by

* However, fatty changes in the liver do not depress albumin levels.

O'Kelly & Seifert (1970), should have revealed significant changes where total serum protein showed only minor shift. The fact that suboptimal levels in serum protein levels were induced (Fig. 6.14, 9.12) emphasizes the drastic effects that accompany heavy infestation. Thus O'Kelly & Seifert (1970) commented that even adequate diet could not counter-balance the depression in growth rate and blood composition caused by heavy tick burdens. The mediation of these low levels could be attributed to blood sucking, interference with liver function and inhibition of the mechanism for maintaining the serum protein equilibrium (Coles, 1974). Nonetheless, a serious liver damage would be questionable since a rapid recovery in both the body condition and blood picture occurred on cessation of infestation. This rapid recovery on the cessation of tick infestation has been observed in other studies (Lutu, unpublished, Philip et al., 1935). At any rate standard liver function tests can be used to establish the involvement of the organ in the emaciation syndrome.

The estimations of serum glucose produced generally high values even in wasted animals and there were infrequent significant differences in the mean values between uninfested and infested calves. This further casts doubt on the significance of liver dysfunction in the disturbed body metabolism and deteriorated physique normally associated with deficiency in energy metabolites. However, investigation of serum glucose reaction was speculative as the literature reviewed scarcely

mentions response in the index to tick effects. O'Kelly & Seifert (1969) found no changes in the serum glucose levels of tick infested cattle. The results from my study do support the findings of O'Kelly & Seifert (1969) and thus indicate that the serum glucose index is stable in the bovine. Nonetheless, the high glucose levels obtained could be related to samples taken without starvation, as Coles (1974) states that the blood values of young, as opposed to adult, ruminants are influenced by feeding.

The sensitivity of the biochemical parameters to tick effects, which was generally low, does not permit precise assessment of the role they played in the development of the wasting syndrome. Therefore more tangible responses in blood composition would warrant testing more sensitive indices e.g. albumin, serum glutamic oxaloacetate transaminase (SGOT), cholesterol. As stated before, the role played by circulatory derangement was less obvious in the calves as opposed to guinea pigs. It is not certain whether tick effects were severe enough in calves to cause impaired circulation that would inhibit normal metabolic processes. In contrast, the guinea pigs developed massive haemorrhages that diverted the nourishment from the normal destination in the body. Nevertheless, the rapid death that followed did not allow development of frank changes related to wasting. Thus it is considered that the rapid death, in the absence of exsanguination, arose from a combination of antigen-antibody by-

products and direct effects of massive infestation. Debility that appeared without apparent significant changes in circulation and blood composition leaves room for postulation of intoxication which is still undefined.

Pyrexia was a prominent feature in the syndrome(s) related to the infestations of R. appendiculatus and its occurrence over prolonged periods does disturb the general body metabolism. The sequelae to the disturbed metabolism would include loss of body condition. This is mediated through tissue breakdown (protein, fat) and uncontrolled excretion of soluble metabolites (proteinuria) which deprives the body of sustenance (Coles, 1974). The situation inevitably would lead to wasting and loss of general body condition. During this study the effects were evident in the successive infestations of high density adult and concurrent three-instars of R. appendiculatus. The animals with pyrexia lost condition rapidly and in addition proteinuria was found in the few samples analysed. However, injury to renal tissue from other causes, even direct toxicity from tick secretions, can also lead to escape of metabolites. Renal dysfunction is a very possible contribution as it was often found that the kidneys were enlarged and haemorrhagic in guinea pigs which died. In the course of tissue breakdown other physiological functions are deranged making the patient physically weak, feed little or not at all which accelerates wasting.

Parenchymatous organs, e.g. liver, may also suffer some degeneration which reduces efficient function. It is interesting to note that mean pyrexia levels regressed with time to the base which indicates that ^{the}wasting syndrome did not owe much to pyrexia effects during the latter infestations. Thus the progressive decline in condition of calves was attributed to other factor(s). Once again the guinea pigs died quickly before marked changes in the condition were developed. Nonetheless, marked wasting occurred in some of the guinea pigs which sustained pyrexia for a long time after the cessation of infestations. It is noteworthy that pyrexia is often accompanied by dehydration which would severely strain body function and exacerbate the intoxication from specific effects if unattended. Another sequel to pyrexia is anorexia and this did not develop in calves but appeared in guinea pigs. Thus loss of condition in calves at most times was not dependent on pyrexia or pyrexia induced anorexia. However, it is not clear whether anorexia in guinea pigs was associated with pyrexia since it occurred in cases where the body temperature did not rise at all during tick exposure. It could be suggested therefore, that pyrexia in calves exerted its effect through tissue breakdown and inhibition of normal function of organs (liver, kidneys).

Having commented on the role of pyrexia in the wasting syndrome it would then be appropriate to examine its development and the pattern it manifested. In

general pyrexia levels emerged at the active feeding phase of the ticks during the primary infestations showing a monophasic peak which coincided with period of engorgement. In contrast, repeated infestations induced biphasic pattern peaks with the earlier one appearing well before engorgement, indicative of hypersensitivity reaction. The adult infestations induced more consistent reactions as opposed to the immature instars where much lower degree and infrequent levels obtained. The difference between the severity of pyrexia produced by the adult and immature instars could be associated with volume/concentration of secretions injected into the host. This assumes that the adults secrete a much greater volume of saliva than ^{the} corresponding number of immature instars which are much smaller in size and thus with lower feeding capacity. However, variation in composition of secretion could also be contributory (Howell, 1966; Neitz et al., 1969; Tatchell & Binnington 1973). The pyrexia as obtained during the primary experiments were confirmed during the secondary experiments (C.10.2.1). My findings corroborate the observations made by Wagland (1975) on varied response to first and subsequent exposures to Boophilus microplus. Wagland found ^{that} the larval phase did not provoke pyrexia on first exposure which agrees with the apparent lack of rise in mean rectal temperature to pyrexia levels during the infestations of R. appendiculatus larvae. He related the earlier rise in the diphasic pattern to sensitization.

This is corroborated by Aitkin (1960) who demonstrated that a hypersensitivity state induced elevated body temperature on challenge. That this pyrexia reaction to R. appendiculatus is distinct from fever caused by infectious agents was supported by van Rensburg's (1959) report of raging fever and apathy in healthy calves massively infested with Boophilus decoloratus. In this study it was generally found that the pyrexia disappeared with cessation of infestation. This timing further shows that the reaction is of a non-infective nature and thus differs from the fever pattern produced by tick-borne infection demonstrated by Brocklesby (1962). Moreover, antibiotics administered during the secondary experiments failed to inhibit the development of pyrexia on infestation. Nonetheless, failure to respond to antibiotics does not specifically eliminate other insensitive pathogens, such as Nairobi Sheep disease (NSD) virus infection, transmitted by R. appendiculatus, but this is accompanied by classic systemic symptoms unobserved in this study. At any rate NSD virus does not produce significant clinical responses in cattle. It is worth noting that the uninfested control calves developed fever of non-specific origin during the primary experiments. This anomaly in the controls was eliminated by the consistent pyrexia pattern induced in the infested calves. Elucidation of the nature of the fever produced by R. appendiculatus could also be made by passage of blood from pyrexia cases into susceptible hosts. In true

infection there must be a prepatent period before the typical fever pattern appears. However, this approach was not undertaken. During this study secondary infections caused complications in the pyrexia pattern. In one case which developed pneumonia the antibiotic therapy could not control the pyrexia. In retrospect the cause was chronic bronchopneumonia of Corynebacterium pyogenes which resists the drug effects on account of the inflammatory changes which shield the organisms. However, were these complications to occur frequently the responses to specific tick effects would be seriously overshadowed. The differential signs associated with such infections should assist in the diagnosis of the cause of the observed pyrexia. In this case there was unremitting fever, coughing, neutrophilia and terminal dyspnoea. In the absence of distinct secondary infections, diagnosis of pyrexia associated diseases under field conditions should therefore consider the tick effects where R. appendiculatus infestation exists. However, knowledge of other clinical signs associated with infestation of Rhipicephalus sp. would be of great assistance. This observation should serve as a reminder of the recommendation made by van Rensburg (1959) that diagnosis of Rhipicephaline toxicosis should be based on a herd unit and not on individual animal which can suffer obscure conditions.

Though pyrexia generally disappeared on cessation of infestation there were definite cases where it persisted beyond detachment of engorged ticks. This

appeared fairly often in guinea pigs and it could be associated with persistence of tick secretion agents in host tissues. The possibility of residual pyrogens in tissues is analogous to the development of tick paralysis in calves after the causative tick, Ixodes holocyclus, had detached as described by Doube & Kemp (1975). The regression against time in the mean pyrexia levels was a prominent feature and the regression was significant in one group (Fig. 5.1; $r = -0.86$ a.m.; -0.89 p.m.; $p < 0.001$). It would therefore appear ^{that} the body becomes immunised against tick pyrogenic agents, thus inhibiting temperature derangement or, alternatively, the high concentration (cumulative effect) of the agent(s) paralyses the heat-regulating mechanism preventing further responses. Conversely, suboptimal rectal temperatures in cases which eventually succumbed could be attributed to weakened state of body metabolism that depresses brain and circulatory activity. However, such temperatures were also recorded in animals not showing extreme weakness (Fig. 5.1). The concept of paralysis of the heat regulating mechanism is supported by lack of shivering in hosts that developed subnormal rectal temperatures. Shivering should have appeared in hosts, especially guinea pigs, which rapidly succumbed to massive R. appendiculatus infestation. The subnormal temperature appeared very early in infestations lasting two to three days before death. It is interesting to

note the difference between groups (II) and (III), both infested with adult ticks, which can be closely associated with density and interval of infestation. Similar patterns of pyrexia appeared under the two regimes but the group (II) showed significant regression as opposed to group (III) (high density infestation) which showed lower pyrexia levels and no significant regression. Thus the high density infestation exerted greater inhibitory effect on the mean pyrexia response which could be attributed predominantly to direct intoxication and to a less extent on an immune response. The development of subnormal temperature, unpreceded by pyrexia, during the first infestation in guinea pigs further supports the concept of general intoxication from R. appendiculatus secretions. On the basis of these observations it would therefore be reasonable to suggest that the pyrexia induced was due to specific effects in primary and successive exposures and hypersensitivity reaction during latter exposure.

Several workers (Feder, 1944; Earle, 1946;

Neitz, 1962, Riek, 1956; van Rensburg, 1959) made casual reports on temperature elevation associated with tick infestation but in a number of cases such reactions were confounded by concurrent infestations. Aitkin (1960) describes causes of non-infectious pyrexia, e.g. pharmacological agents, venom and endogenous toxins. The endogenous toxins are related to injured white blood

cells which release pyrogenic substances that stimulate the hypothalamus. Various workers, such as, Schleger et al., (1976); Tatchell & Moorhouse (1968); Berenberg et al., (1972), demonstrated massive infiltration of tick bite wounds with leucocytes that appeared degenerate or degranulated. This observation concurs with the theory described by Aitkin (1960) but the concept becomes less tenable in respect of infestations of immature instars of R. appendiculatus which seldom provoked pyrexia despite severe local reactions (hypersensitivity). Wagland (1975) detected temperature rise when B. microplus adults and nymphs commenced feeding but not during larval feeding when animals were exposed to first infestation. Lack of sufficient data on the dynamics of leucocytes during tick infestation is a limiting factor in interpreting the pathogenesis of associated fever though Williams et al., (1978) stated that specific tick effects depressed peripheral blood leucocyte levels. The depressed levels could be associated with massive tissue infiltration (skin layers) and/or depressed bone marrow activity. Thus the pyrexia induced by Rhipicephalus appendiculatus infestation can be attributed to specific effects and by-products of salivary secretion and host tissue interaction, e.g. histamine and related pharmacological agents, but the component of the saliva responsible for the stimulus is undefined.

In review, the causes associated with loss of body condition during this study can therefore be summed up as worry, anaemia and suboptimal serum protein levels, pyrexia and reduced feed intake, all working in consort. The role played by each varied between groups and individual animals. However, these features on their own do not complete the debility picture and therefore in the absence of better explanations it can be suggested that more subtle changes were induced through progressive tick secretion effects. Removal of tick challenge was followed by rapid recovery in both muscle tissue and blood composition of the calves, thus showing the regenerative potential was not completely denatured. However, guinea pigs showed slow recovery in clinical condition.

11.2.2. Dysfunction in the digestive system would be a significant factor in the wasting syndrome as all nourishment from the ration has to pass through the digestive tract. In general calves sustained much less disruption in the normal function than the guinea pigs under stress of R. appendiculatus infestation. Thus the contribution of impaired function of the system toward the debility in calves was less prominent. It is interesting to note that the calves maintained appetite practically to the end as they could still eat during extreme weakness in terminal stages if the feed was close to their head. However, as prostration developed there was appreciable reduction in the amount, especially of bulk feed, eaten. This was basically associated with

sluggish mode of eating i.e. prehension, mastication. The digestive tract functioned normally though some of the guinea pigs suffered complete suspension of activity. This anorectic response was attributable to direct tick effects, though pyrexia could have been contributory where it occurred. It is noteworthy that calves seldom passed constipated faeces which normally appear in infectious conditions causing fever and dehydration. The overall normal function of the digestive system in calves discounts the association of defective digestion/absorption with the debility syndrome. The progressive wasting in the absence of pyrexia, anorexia and dysfunction of the digestive tract strongly indicates that the cause lies in the specific tick effects which probably interfere with the assimilation of absorbed nourishment in the body tissues. O'Kelly & Seifert (1969) found that heavy infestation significantly affected feed intake which was accompanied by failure to grow or gain weight. They also demonstrated that a high quality ration has an ameliorating effect on the response to tick infestation. However, O'Kelly & Seifert (1970) asserted that the resilience in response (resistance) in animals adequately fed can be overcome by massive infestation. Thus the effects observed under infestation of R. appendiculatus were not predominantly dependent on digestive system dysfunction. It is often observed in the field that cattle infected with Theileria parva (causing East Coast fever) maintain appetite until late in the course of the

disease, and this feature occurred in this study. Thus appetite as a differential feature cannot be dependable during the early course of rhipicephaline syndrome(s) and I. parva infection. It is important though to consider entire body system responses instead of concentrating on an individual parameter, unless it is pathognomonic for the particular condition. Besides, the effects of tick infestation and I. parva infection can easily develop concurrently where cattle are not regularly sprayed. During this study an unusual response developed in some calves, which produced copious saliva under challenge of active tick feeding. The response appeared under both adult and immature instar infestations. It was an interesting observation, as clinically there was no apparent lesion detectable in the mouth or related head structures that could be implicated. Excessive salivation occurs in various infectious diseases e.g. foot and mouth disease, or chemical poisoning. In the latter case there may not be any suggestive evidence of the cause in the mouth. Organophosphorus compounds used for tick cleansing often cause accidental poisoning but the salivation is accompanied by other nervous signs. Salivation is associated with stimulation of the parasympathetic autonomic nervous system and this stimulus need not arise from pathological lesions in the mouth. The coincidence with the active phase of tick feeding strongly indicates that the stimulus is mediated through the injected tick secretion or a by-product of an interaction between secretion and host tissues. This suggestion would be in

accord with observations made by Tatchell (1969a) on the host-parasite relationship. He asserted that the manifestations in the host could be mediated through direct tick secretions or complexes formed by antigen-antibody reaction whereby active pharmacological substances are released in the body. It would be interesting to find out whether this response is dependent on the undiluted R. appendiculatus salivary secretion. If the suggestion made on possible stimulus is true it is then difficult to explain why only a small proportion of all animals treated similarly developed the salivation. Thus it gives the impression of an idiosyncrasy or high sensitivity in the affected calves. However, the salivation in the few calves could be attributed to differences in the response of individual hosts to tick agents, as reported by various workers (Howell, 1966; Riek, 1956). Whereas excessive salivation, associated with the tick paralysis syndrome, is described by Gregson (1973), it is noteworthy that this reaction is not reported in other tick-bovine studies reviewed. The occurrence of this response can be of significant clinical importance on the digestive system function where continuous R. appendiculatus infestation exists. Profuse salivation, where saliva drools, can deprive the body of appreciable amount of electrolyte required for digestive processes. The guinea pigs did not evince signs of copious salivation. Thus the two host species showed different clinical manifestations in the digestive system whereby the guinea pigs sustained severe anorexia and bowel stasis. The calves did not show clear cut anorectic response.

11.3. Lymphadenopathy was a significant clinical manifestation especially during infestations of adult R. appendiculatus instar. The relevance of this response lies with the need to differentiate other conditions which provoke lymph node enlargement and complications which arise from the infection with secondary bacterial organisms. Theileria parva and Babesia sp., which are tick-borne, cause lymph node enlargement but, occasionally, field diagnosis is confounded by cases revealing no parasite in blood or lymph node preparations despite development of pyrexia in addition. The situation is made more obscure if the tick infested sites were dispersed over several different parts of the body, thus simulating generalised lymphadenopathy seen in e.g. Theileria parva the infectious conditions, / However, the preference of ear and other head sites by the adult R. appendiculatus would limit enlargement to head lymph nodes which helps to differentiate uncomplicated tick effects from systemic infections causing generalised lymphadenopathy. The enlargement associated with tick infestation was observed by Hewetson (1971, 1972) and van Rensburg (1959) but in the latter case the reaction was complicated with tick-borne infection. Another differential feature related with changes in lymph node reaction was the unresponsiveness that developed on repeated infestation. This unresponsiveness was reported previously by van Rensburg who attributed it to necrosis of lymphoid follicles. It

is interesting to note that the size of grossly enlarged nodes regress in long standing cases of I. parva infection in cattle. While the dynamics involved in this regression differ between the two conditions it is worth recognising as diagnosis of I. parva infection based on lymph node puncture preparations from such animals may give inconclusive results because of the depleted lymphoid cells. The conclusions made by van Rensburg (1959) are further supported by Neitz (1962) description of leucocytotropic toxin in the R. appendiculatus salivary secretions. It can then be suggested that the unresponsiveness and induration of lymph nodes of repeatedly infested calves was caused by specific tick effects. Exploratory centesis and autopsy cut surfaces revealed haemorrhagic and coagulative degeneration in nodes draining infested sites which would culminate in destruction of the lymphoid follicles. The haemorrhagic changes have to be closely examined if found in several superficial lymph nodes as they do also occur in severe I. parva infections. However, in cases of uncomplicated acute rhipicephaline toxicosis generalised haemorrhages occur (Dr.M.P. Cunningham, pers. comm.). Having made the above observations I would state that gross changes occurred only in the lymph nodes draining infested areas in my calves. On the other hand the guinea pig model presented a markedly different picture. During massive primary and repeated exposures inflammatory changes developed in both superficial and deep lymph nodes. The vascular changes can be specifically induced by tick

secretions (Tatchell & Binnington, 1973; Tatchell, 1969a; Howell, 1966). The generalisation could be attributed of tick infestation to small body size relative to density/ and the secretions of the applied ticks. The vascular changes in the repeated exposure were partly attributed to immune responses releasing substances e.g. histamine, heparin, that cause dilatation and leakage. Whilst the progressive changes induced in the local drainage lymph nodes are not clearly defined it can be suggested that sequential biopsies taken at different stages during exposure and after would help to elucidate the response of the nodes. However, biopsies have to be taken from different nodes each time in order to avoid artefacts caused by surgical trauma.

11.3.1. Lymphadenopathy observed during infestation of R. appendiculatus was not of infectious origin, though secondary bacterial infection produced persistent enlargement before the abscesses burst spontaneously. The secondary complications would arouse doubts on the development of the reaction in ^{the} absence of infection. However, during secondary experiments some of the infested calves were given prophylactic antibiotics in therapeutic dosage to control possible infection. I found then that the treated calves developed enlargement in drainage nodes, without abscess complication and that the size reduced rapidly on cessation of infestation. In support of these observations Gaafar (1972) states that enlargement of lymph nodes in specific response to arthropod

is stimulus/mediated through oedema and enlargement of lymphoid follicles. Oedema increases as a result of the greater lymph drainage from the inflammatory area. The inflammatory area carries high concentrations of leucocytes including macrophages (Schleger et al., 1976; Wikel et al., 1978) which are conveyed to the local lymph node through increased lymph drainage, thus distending the size of the node. It has been observed that development of wounds from tick bites depends on the infiltration of neutrophils (Tatchell & Moorhouse, 1970) and their migration is influenced by complement-derived chemotactic factors (Berenberg et al., 1972). Theis et al., (1976) consider migration of the macrophages through inflamed sites (tick attachment areas) into local nodes can serve as a vehicle for the spread of transmitted infection. Thus the increased lymph flow to and through lymph nodes exposes the body systems to widespread infection as the lymph finally drains into the main blood stream. The infection arising from bite wounds is generally pyogenic in nature in the absence of the recognised tick-borne diseases. Several previous workers have reported abscessation in the local drainage lymph nodes associated with tick infestation (Thomas & Neitz, 1958; van Rensburg 1959; du Toit & Theiler, 1964) and Dr. Taylor (cited by Hoogstraal, 1956) reported involvement of Corynebacterium pyogenes. du Toit (1964) & Theiler/described suppurative reactions in deep tissues. The pyaemia in lambs associated with tick bite could have

developed through lymphatic spread. Thus these findings are in conformity with the results obtained in the present study where C. pyogenes infection spread from lymph node abscesses to the lungs, causing bronchopneumonia. The relationship between lymph node abscess and pneumonia was revealed by the identical bacteriological isolation of C. pyogenes between the two sites. However cases of abscessation of lymph nodes were diagnosed in calves which had no distinct clinical signs of pneumonia but at autopsy there was evidence of ^a/suppurative reaction in the lungs. The inhibition of clinical pneumonia signs could be attributed to the immediate withdrawal of the animals from the experiment which reduced the aggravating stress from tick infestation before they were slaughtered. The vulnerability of the pulmonary tissue to such infection as described by Jubb & Kennedy (1970), explains the sequence of events culminating in pneumonia. It should be pointed out, however, that the first pneumonia case was not associated with lymph node abscess until the autopsy findings were analysed. Thus tick bite wounds form portals of entry for pyogenic organisms which cause serious systemic infections. This situation possibly occurs often under field conditions where tick cleansing is inefficient and would cause serious economic losses. It need not be emphasized that chemotherapeutic treatment of such cases affected with pyogenic organisms will not be economic because of the nature of the lesions produced that impede diffusion of the drugs. It is noteworthy that Corynebacterium spp. can occur commonly on

bovine skin and would thrive under reduced oxygen tension environment as offered by the necrotic tissues around tick wounds. These properties pose a great threat to animals infested heavily with ticks. Therefore it can be concluded that alteration in lymph draining infested sites as it passes through the local node(s) predisposes the host to serious secondary bacterial infection.

11.3.2. The speculated lymphocytolytic effect associated with lymph node changes (Neitz, 1962), though unproven, could also be applied to the shrinkage of the spleen in infested calves. The autopsies on the calves revealed characteristic splenic changes which could be associated with the wasting syndrome. The shrinkage appearance simulates the status created by splenectomy when operated animals become more susceptible to tick-borne diseases, e.g. anaplasmosis. van Rensburg (1959) suggested that the increased susceptibility to tick-borne infections in bovines heavily infested with Rhipicephalus spp. was caused by depression of the reticuloendothelial system defensive mechanism. In his studies he recorded moderate to severe atrophy of the spleen in steers infested with R. appendiculatus. It is interesting to note that atrophied spleens were found in animals which were in good condition. In assessing these findings he revealed that such animals had experienced moderate to severe exposure to R. appendiculatus though effects from other tick species which appeared concurrently could not be eliminated. It would therefore be logical to suggest

that the increased susceptibility to tick-borne diseases (1959) observed by van Rensburg was attributable to spleen atrophy. In view of these findings it can be further suggested that the splenic hypoplasia induced in calves during this study was caused predominantly by specific tick effects as opposed to ^{the} general wasting syndrome. In contrast the spleens of the guinea pigs were generally thick and dark in colour which could be attributed to species difference in response to tick effects. It would be interesting to test the hypothesis advanced by van Rensburg on the inhibition of the reticuloendothelial system function in animals infested with R. appendiculatus. Such studies should include tick-borne disease challenge in susceptible bovines which had been exposed to repeated moderate infestation of R. appendiculatus. This should be compared with controls infected with tick-borne disease(s) without tick exposure.

11.4. The dermatitis reactions mediated through wounds, necrosis and hypersensitivity reactions confirmed the features demonstrated by many other workers, such as, Hewetson (1971, 1972), Riek (1962), Thomas & Neitz (1958) van Rensburg (1959), Hewetson & Nolan (1968). The more exaggerated reactions during secondary exposures are attributable to by-products of immune responses (Tatchell & Moorhouse, 1968; Allen & Wikel, 1978; Allen, 1973; Riek, 1962). Riek (1962) and Tatchell & Bennett (1969) revealed reactions that represented inflammatory changes in previously sensitized animals and these were connected

to a great extent with histamine and allied substances. Histological studies helped to elucidate the pathogenesis of the gross clinical manifestations (Allen, 1973; Schleger et al., 1976; Tatchell & Moorhouse, 1968). Cellular infiltration in the parasitised areas were more dense during secondary exposures. The specific cells had different roles to play, i.e. basophils were associated with histamine release that mediated dropsical (blebs) changes, and neutrophils were associated with necrotic changes at the bite wound. Tatchell & Moorhouse (1970) describe the close connection between neutrophils and development of bite lesions, suggesting^{that} the lysozymes released by degenerate cells mediated the necrosis and this observation was corroborated by Berenberg et al., (1972). Thus the degeneration of skin layers could result from direct saliva effect on the cells or indirect blockage of capillaries with sequestered leucocytes, causing infarcts. The increased permeability of vessels leads to exudation and oedema. In my study the oedema of varied degrees accompanied by exudation, and bleb formation and persistent erythema was commonly produced during different infestation regimes. In contrast the predominant lesion exhibited by the guinea pigs was persistent erythematous spots long after all ticks had detached. Whilst the adult instar inflicted the most serious wounds it was interesting to note that the larvae provoked the most marked clinical hypersensitivity reaction. The induced blebs were so severe that

virtually all applied larvae applied were annihilated at the time. Under field conditions such reaction would cause marked reduction in the viable tick population. My findings confirm the responses demonstrated in bovines (Riek, 1962) and guinea pigs (Allen, 1973). Allen working with Dermacentor andersoni found this hypersensitivity state was associated with massive infiltration of ^{the} skin layers by basophils and eosinophils. The granules of the basophils carry histamine (Kemp, 1978). The sequelae to repeated exposures in calves were associated with peculiar pitted scars simulating aftermath of pox virus infection. They remained prominent for a long time. However, the pathogenesis of the lesion associated with the scars is not clear as they were not found in all previously sensitized animals nor on primary infestation. During primary experiments previously exposed animals reacted more severely and also manifested lesions undetected on first infestation. These observations were confirmed during the secondary experiments. Distinct necrotic and exudative changes involving deep layers of the skin occurred on repeated exposure whereas the first exposure produced superficial necrosis and less exudation. It could be suggested that the difference was dependent on the higher concentration of neutrophils though it is doubtful that lower infiltrations developed in others which did not show such scars. On the basis of histological evidence (Tatchell & Moorhouse, 1968) it could be further

suggested that the depth of the lesion was influenced by severe Arthus-type reaction (Herbert, 1970), which on resolution formed a sit-fast necrotic plug followed by the scar. These scars were also reported by Pavlovsky & Alfeyeva, (1941); Riek, (1962); Hewetson & Nolan, (1968); Hewetson, (1971).

10.4.1. Alopecia, an important differential feature, was commonly produced around infested sites on the ears of calves and backs of guinea pigs. A similar reaction associated with tick feeding was reported by Wilkinson (1955), Philip et al., (1935) Gebelhoff (1973). In the absence of excessive inflammatory reaction and exudation (accompanied by encrustation) the cause of depilation is difficult to determine. It appears that the hair shafts are completely removed as there was no evidence of the stubbles seen in ringworm infection and yet regrowth of hair developed rapidly after infestation. Nonetheless, Whitwell (1978) found that the hair shaft resisted tick secretion effects and that after removal of the stimulus the hair follicle cells produced new cells that formed the new hair. Despite this revelation the mechanism underlying the loss of existing hair remains undefined. In view of the clinical-histological evidence it can be suggested the depilation is mediated by a specific agent in the tick secretion or by-product of secretion-body tissue interaction in the absence of physical suffocation from excessive exudate/encrustation. Marshall (1966) suggested ^{that}/heparin and related substances caused depilation.

It is possible that such substances occur in the tick secretion or are released by injured cells (Tatchell, 1969a). As the composition of the R. appendiculatus secretions is not known the speculation may not be tenable. However, prolonged bleeding from bite wounds noted in the present study would support the heparin concept as the presence of anticoagulant would be partly responsible. Partial responsibility is suggested because other causes of faulty blood coagulation e.g. thrombocytopenia, were not tested. Code (1939) found that blood from anaphylactic cases carrying elevated histamine levels showed poor coagulation properties. Thus the mechanism underlying the alopecia associated with R. appendiculatus infestation cannot be clearly explained.

Distortion (curling, crumpling) induced in the ears of calves could be attributed to contracted scar tissue or loss of elastic tissue (Pavlovsky & Alfeyeva, 1941). Clifford (1954), du Toit & Theiler (1964) described sloughing of ears and tails as a direct result of tick feeding. In the absence of secondary necrotising complications such effects could be attributed to lytic activity in the tick secretion as demonstrated by Moorhouse (1968) in the ears of guinea pigs infested with Ixodes sp. Severe oedema of the pinna, simulating a haematoma reaction, can cause shrinkage and distortion on healing as a result of loss of elastic tissue and uneven scar formation. Arthur (1962) describes serious consequences of otitis externa which can

predispose to myiasis/suppuration that is capable of eating away the pinna. Fortunately no cases of myiasis developed in my calves though some suffered severe otitis externa which attracted flies.

11.4.2. It is noteworthy that the lesions induced in the skin resolved rapidly, but the integrity of the epidermis did not stabilise as quickly because dandruff persisted indefinitely. Gaafar (1972) states that the defensive reaction to persistent irritation in the basal membrane of the epidermis is an increase in cell production. These cells may be normal or arrested in development and eventually increase the epidermal thickness. Because of the abnormal position they constantly flake. Thus the same explanation can apply to protracted pityriasis (dandruff) around infested areas that was observed in my calves. Similarly it can be suggested that the chronicity of the condition indicates persistence of stimulus left by thick secretion. This suggestion would be in accord with observations made by Moorhouse (1966) and Doube et al., (1977) that the residual effects of Ixodes holocyclus caused tick paralysis some days after detachment of the tick.

11.5. Repeated infestations of R. appendiculatus instars induced manifestations which were not observed in the primary exposures. Their onset and characteristics simulated a hypersensitivity state. Thus the tick secretions could be described as antigenic. The immunological basis of responses to different tick species in farm and

laboratory hosts has been demonstrated by many workers (Allen, 1973, Wikel & Allen, 1978; Riek, 1962; Roberts 1968a; Willadsen et al., 1978). In addition to in vivo and in vitro tests, immunosuppressants have been extensively investigated in the elucidation of the phenomenon (Allen, 1973; Wikel & Allen, 1976b; Bagnall & Rothwell, 1974). Correlation of the phenomenon with tick performance has also been extensively investigated but a lot remains to be explained. The influence of the immune status on R. appendiculatus in the present study will be discussed later. The associated clinical responses in my study were rapid bleb formation, transient urticarial eruptions, intense pruritis and other severe inflammatory reactions. The infrequent detection of the uticaria reactions could be attributed to the transient nature and eruption in areas remote from infested sites which made them more difficult to find. It would be relevant to state that other anthropod parasites e.g. Culicoides sp., fleas, do induce identical reactions in farm and laboratory animals (Riek, 1953); Larrivee et al., 1964; Burghardt et al., 1951) such that diagnosis of dermatitis in the field should be more broadly based depending on locality and season. Where the parasites are elusive, epidemiological knowledge of the suspected causes can assist in deciding the diagnostic approach. Allergic tests have commonly been used to establish diagnosis under such obscure situations

(Riek, 1954b). The reliability of such tests, however, would depend on the specificity of the allergen used assuming interspecies reactions do not occur. Thus use of arthropod tissue extracts should recognise the marked variation in their composition (Gaafar, 1972) which may greatly influence the interpretation of the responses. In my study, allergic tests, involving the cutaneous Arthus-type reaction (C.A.) and antihistamines were used to demonstrate development of an immune response to R. appendiculatus secretions. The tests provided clear evidence of development of antibodies (IgG, IgE; Herbert, 1970) in both calves and guinea pigs. The passive cutaneous allergic test further demonstrated that the antibodies were transferrable by serum and therefore was humoral in nature. Similar results have been obtained by other workers using different ticks (Riek, 1962; Roberts & Kerr, 1976; Trager, 1939b; Wikel & Allen, 1976a). However, differences between species or individuals in the immunological responses to ticks have been commonly reported (Riek, 1956; Tatchell & Moorhouse, 1968; Howell, 1966), but no elaboration has been made on the possible similarities between immune responses to tick and other arthropods which can obscure diagnosis. This argument relates to possible inter-arthropod species reactions. Analysis of the salivary secretions to determine the constituents would be the answer to this question but collection of sufficient material, especially from smaller arthropods (Culicoides), is not easy.

I found that the immune response, as demonstrated by the cutaneous Arthus type reaction, developed within ten days of first exposure which concurred with observations of Roberts (1968a) and Irvin et al., (1973). On the basis of the rate of reaction in the cutaneous allergic test it was concluded that the manifested hypersensitivity state was of the immediate type though the guinea pigs showed indications of a 'delayed' reaction. However, further characterisation would require other methods of investigation, e.g. histological studies. Though the cutaneous allergic test was done on a quantal basis, it was interesting to observe that the calves showing marked tick rejection had reacted severely to the salivary gland extract (SGE) inoculation. The sequence of events at the test sites was characteristic of an Arthus reaction, i.e. rapid local severe inflammatory changes and exudation, necrosis and sloughing within few days. The relationship between high rejection of ticks and severe cutaneous Arthus reaction would provide a good measure for the degree of the immune response (resistance) induced by R. appendiculatus effects. It would be interesting to find out whether a significant correlation does exist between tick resistance and cutaneous Arthus reaction in bovines exposed to R. appendiculatus. Thus it can be suggested that the severe cutaneous Arthus reaction I observed in the hosts was caused by a high concentration of precipitating antibodies, induced by R. appendiculatus infestations. Besides, the calves developed exudative nodular lesions in attachment areas and contiguous skin which further supports the conclusion on precipitating

antibodies. The persistence and nature of these nodules, before exudation, closely resemble the papules described by Riek (1962). Sloughing of the nodules left crater scars which to some extent would implicate Arthus-type reaction in the formation of the associated lesions described before. However, verification of these changes requires histological investigation. The fact that the incidence was low does require differentiation of other possible causes, e.g. fungal or metazoan parasites (filarial microfilaria). However, none of these were found in these lesions.

11.5.1. Administration of antihistamines in the demonstration of an immune response was based on the fact that histamine is released in increased amounts in the circulation of sensitized hosts when challenged by/^acorresponding antigenic agent. Biochemical estimations (Riek, 1955, 1954a, Code, 1939) and histological investigations (Allen, 1973; Bagnall et al., 1978; Schleger et al., 1976) clearly illustrate the role histamine plays in the mediation of immune manifestations. High serum levels of histamine and massive infiltration of basophils and eosinophils which degranulate have been demonstrated. Massive release of histamine and allied substances cause severe anaphylaxis of local or systemic nature. Fortunately cattle can withstand considerable levels of histamine whereas the guinea pigs are very sensitive (Herbert, 1970). This characteristic is beneficial to the bovine which, under inefficient farming conditions, would

be vulnerable to the effects of persistent challenge of the ticks. Furthermore, this property does explain the difference in efficacy of the antihistamines I administered to calves and guinea pigs whereby in the latter case the drug(s) could not completely protect against R. appendiculatus effects. Riek (1955) found that the elevated serum histamine levels returned to base in about eight hours. Tatchell & Bennett (1969), using different antihistamines/tranquillizers produced a transient inhibitory phase on the host resistance against ticks, which subsequently became boosted. The boosted response was attributed to increased antigenic challenge produced by better performance of the applied ticks, but the performance consequently declined. However, Bagnall & Rothwell (1974) failed to inhibit the resistance to Ixodes holocyclus in guinea pigs using promethazine (antihistamine).

Thus demonstration of the histaminic phenomenon was undertaken during the secondary experiments and the investigations were based on a null hypothesis that, 'the responses induced by R. appendiculatus salivary agent(s) could not be inhibited by antihistamines'. Tripelenamine hydrochloride and promethazine hydrochloride injections were used and they effectively reduced the severity of the clinical signs mediated through hypersensitivity reactions in both calves and guinea pigs, though in the latter case the inhibition was not sufficient in some animals to prevent early death. Thus tick

performance was promoted where it had failed before which partly supports the results of Tatchell & Bennett, (1969). The boosted "immune" response to ticks under medication was not observed because the antihistamines were used for a brief period. The failure in efficacy of the antihistamine in the guinea pig could be partly attributed to direct toxicity from the tick agent(s) and/or the factors released through complex-mediated hypersensitivity (Roitt, 1974). However, autopsy did not reveal typical allergic reaction but there was an extensive haemorrhagic syndrome which could be caused also by intoxication. Since the guinea pig died on the third day of tick feeding, having received treatment for two days, it can be suggested that substances other than histamine played a significant role in causing death. This suggestion would partly explain the failure of promethazine to inhibit the "immune" response in guinea pigs infested with I. holocyclus (Bagnall & Rothwell, 1974). These observations would therefore suggest that the clinical manifestations of resistance to the tick agent(s) are produced by both histamine and other factors released through a complex-mediated hypersensitivity.

The clinical manifestations induced during primary experiments and responses (cutaneous allergic test and antihistamines) demonstrated in secondary experiments clearly show that R. appendiculatus induces immune responses in the parasitized hosts. Thus it would be relevant to suggest that the cellular and humoral components of the immune response demonstrated in other systems can apply to

Rhipicephalus appendiculatus - cattle system (Bagnall & Rothwell, 1974; Wikel & Allen, 1976a).

11.6. The influence of immune responses upon tick performance has been extensively studied (Roberts, 1968^b, 1971; Riek, 1962; Bennett, 1975; Bailey, 1960; Trager, 1939a; Allen & Wikel, 1978; Tatchell, 1969a) and the success of performance has been used as a measure of host resistance (Hewetson, 1971). The characteristics observed in my study which inhibited successful tick performance included pruritis, which evoked/^afrantic desire to groom; severe epidermal reaction manifested by blebs and excessive exudation; and clinically inapparent causes. The inapparent factors were associated with failure to attach when ticks wandered and finally disappeared within twenty four hours. Branagan (1974), Roberts (1971); Tatchell & Moorhouse, (1968); Kemp et al., (1976); Koudstaal et al., (1978); Kemp (1978), observed that ticks took much longer or failed to attach on resistant hosts even in/^{the}absence of frank skin reaction. I found that rapid development of excessive exudation and bleb formation impeded successful attachment, especially of larvae. Detachment and migration to new sites occurred or ticks were engulfed or remained anchored in the pellicle of the blebs and withered and eventually flaked with peeling pellicle. Delayed attachment caused exhaustion followed by dropping off. This exhaustion, especially of larvae, could have been caused by desiccation over/^{the}skin surface (Roberts, 1971) The

clinical hypersensitivity effects observed corroborate the findings of other workers such as Allen & Wikel (1978), Branagan (1974), Riek (1956, 1962), Hewetson & Nolan (1968), Hewetson (1971). However, all sensitized calves in my study did not evince such severe reaction, consequently the wide variation in tick recovery rates and engorged weights from individual hosts. The individual differences in resistance are recognised (Francis, 1966, Riek, 1956, Seifert, 1971). The differences in the responses of calves and guinea pigs I observed are supported by studies made by Branagan (1974) and Riek (1958). Pruritis, which was constantly induced, led to vigorous grooming whenever restraint slackened and thus the behaviour dislodged and/or crushed the attached ticks. It was found that calves, lying down, rubbed their head side/ears on the floor in the absence of standing objects in order to relieve the irritation. This observation supports Koudstaal et al., (1978), Snowball (1956), Kemp (1978) who emphasised the influence of grooming on tick survival. I found that the urge to groom abated soon after the engorged ticks had detached which agrees with findings of Koudstaal et al., (1978) and Hewetson (1971). However, guinea pigs showed impulsive grooming, even on the first day of tick application, which obscured the assessment of pruritis attributable to an immune response. Their sensitivity again made them groom for much longer after cessation of infestation. Thus, pruritis induced in both host species was a significant

manifestation of the R. appendiculatus - host (calves) interrelationship. Therefore in the absence of the ameliorating effect of antihistamines the survival of the applied ticks depended on/use of proper restraint devices. Besides, restraint also permitted development of tangible clinical-pathological responses which would have been inhibited if grooming occurred.

Besides the influence of ^{the}/above effects on tick performance I found that a proportion of successfully attached ticks died in situ before repletion or fed normally to repletion but died on detachment. These findings agree with studies made by Bennett (1975), Koudstaal et al., (1978), Francis, (1966), Riek (1956), Doube & Kemp (1975), Roberts (1968b) whereby death of attached ticks continued throughout the life cycle. Tatchell (1969b) similarly reported death after normal engorgement in Ornithodoros savignyi fed on immune rabbits. I found also that some batches of engorged instars fed on repeatedly infested calves failed to moult. Guinea pigs, in contrast, had a drastic effect on fed females which remained stunted in situ or rapidly faded in colour, withered and died on detachment. Consequently a high percentage of the engorged females, irrespective of exposure, laid poor or no eggs and their hatchability was equally poor. Thus the guinea pig exerted a much greater inhibition on tick performance than calves and was therefore unsuitable for rearing breeding ticks. In support of this conclusion Riek (1959) found the species unsatisfactory during tick studies involving laboratory

animals. Other features associated with measurement of host resistance included the recovery rates of engorged ticks and their weights, and reproductive index and fertility of eggs. However, I did not find a consistent decline in the enumerated features in ticks fed on calves. The divergence from observations made by other workers (Branagan, 1974; Irvin et al., 1973, Bailey, 1960) is illustrated in Fig. 3.6 representing mean engorged weights of adults obtained from successive infestations. According to these workers a significant regression in the mean weights is expected with successive infestations reckoning the effects of immune responses increase with repeated exposure. However, in my results, instead of progressive decline the trend reversed in the latter infestations. The anomaly cannot be explained clearly though a similar phenomenon has been reported in other studies involving other tick species. Unfortunately there are no comparable figures for R. appendiculatus fed on cattle for protracted periods. Though Branagan (1974) noted^a/decline in mean engorged weights of R. appendiculatus during East Coast fever transmission experiments the regression was not significant. The data on the mean weight of R. appendiculatus fed on immune cattle systematically infested is limited and offers no scope of drawing definite conclusions. As a comparison, Wagland (1975) exposed previously unsensitized Brahman and Shorthorn cattle to four infestations of

B. microplus larvae. The mean weights recorded for each infestation between the two breeds showed similar trend to my findings. The anomaly can be related to variation in levels of resistance in individuals, which would influence the degree of engorgement of ticks. Thus with low resistance the ticks would attain greater engorgement and engorged weights, as opposed to situations involving high resistance levels. If proper engorgement influences the final weight then the reversed trend shown in Fig. 3.6 should not be unexpected as on many occasions the recovered ticks appeared normal in size. Besides, the individual clinical responses, if they can be taken as a measure of resistance, varied from mild to severe which should influence the mean values of the recovered ticks. The validity of the observed trend is supported by studies of Bennett (1975), Kemp et al., (1976), Hewetson (1968) Hewetson & Nolan (1968) who demonstrated normal engorged weights of ticks fed on resistant cattle. Thus Bennett showed that host resistance did not impede repletion once the attachment of the instar is established. Similarly, Kemp et al., (1976) found that larvae applied to highly resistant animals showed slower growth rate initially but subsequently attained the normal engorged weight. Furthermore, Gladney et al., (1973) asserted that the nutritional state of the host influenced the burden and the engorged weights. Thus hosts in poor state were liable to carry heavier burdens with high engorged weights, and this may

be attributed to the weakened defensive mechanism. In my experiments the rise in the mean engorged weights occurred when the calves showed a marked decline in body condition. It appears from the above observations that the crucial factor for engorged weights of ticks applied to immune cattle is established feeding. Whilst ticks fed on rabbits show significant decline in engorged weights the cattle fed ticks need not follow the same trend relative to resistance levels acquired in the two species. It is unlikely that rabbits which develop much higher resistance, will yield any sizeable ticks after three successive infestations, whereas the majority of my calves continued to yield engorged ticks after the fifth exposure. Thus cattle can be described as more natural hosts for R. appendiculatus than rabbits which agrees with the assessment of the bovine as the most satisfactory host (Branagan, 1974; du Toit & Theiler, 1964; Irvin et al., 1973). In view of the small numbers of animals used and lack of adequate data on engorged weights of R. appendiculatus fed on immune cattle this anomaly should not be critically analysed.

Ticks affected by host immune responses are recognised by the various characteristics mentioned above, however, the mechanism underlying the disturbance of tick physiology is not clear. Nonetheless, various workers have advanced different explanations for this phenomenon, i.e. effects of host reaction and tick physiology (Allen, 1973, Trager, 1939a, Doube & Kemp, 1975), imbibed host factors, alteration of attachment site creating unsuitable feeding

conditions (Roberts, 1968b). The imbibed host factors were investigated by Bagnall & Rothwell (1974), and Kemp (1978) whose findings involved histamine effects. Bagnall & Rothwell (1974) found that ticks died after ingesting basophil/eosinophil granules. Blood stained faces were passed by these ticks, indicative of a deranged digestive process. The in vitro test on histaminic effects studied by Kemp (1978) showed that once the attachment is established the histamine was not effective.

Another defensive mechanism described involved walling off of the hypostome by the host epidermal cells, thus starving the tick as nourishment is cut off (Francis, 1966; Rubaire-Akiiki, 1977; Trager, 1939a; Berenberg et al., 1972; Tatchell & Moorhouse, 1970; Riek, 1959). The analogy to walling off observed in my study was ticks anchored in moist necrotic epidermis separated from the underlying tissues. The ticks were therefore deprived of nourishment through necrosis and separation of tissues at the attachment sites. Such ticks progressively withered in situ and eventually flaked with peeling epidermal epithelium. In some cases encrustation of excessive exudate physically immobilised the ticks, preventing insertion of the hypostome to an appropriate depth for proper feeding. It is noteworthy that all three instars showed fairly constant feeding periods to engorgement during repeated infestation in contrast with observations made by Branagan, (1974).

11.7. Appraisal of the clinical-pathological syndromes induced by infestations of Rhipicephalus appendiculatus applied to calves reveals reactions which were inconsistent with the general responses. These atypical reactions which strongly indicate intoxication effects are best illustrated by the episode of haemoglobinuria of unestablished origin that occurred in two calves during the primary experiments. The dramatic reaction was accompanied by critical depression in erythrocytic indices, body temperature and general circulatory function. Close examination revealed no erythrocytes in the urine nor haematogenous parasites responsible for intravascular haemolysis. The reaction occurred after repeated infestation and did not recur in the affected calves. The cause was not clear as other possible causes, e.g. leptospirosis, copper poisoning, did not match the syndrome as described in C.6.1. Copper poisoning would affect more animals as the water and feed had a common source and enteritic signs were not found. Leptospirosis was ruled out on lack of temperature and jaundice and appearance of individual case in confined quarters. Further laboratory tests were not indicated because of the pattern of the episode. Occurrence in previously sensitised animals arouses suspicion of an antigen-antibody mediated reaction. But this is unlikely as the percentage affected was extremely low, discounting cases possibly missed in the males which were not always seen urinating. Nonetheless, the

dramatic circulatory changes provoked at three days of infestation are suggestive of massive injury which is not characteristic of anaphylaxis. The other possible explanation is direct effect of tick secretion, though it becomes less tenable as the reaction did not appear in calves which carried greater tick burdens. This is further weakened by lack of such reaction in guinea pigs which were submitted to massive infestation. The guinea pigs sustained generalised severe haemorrhages in different tissues. The low incidence and lack of literature on this response in animals heavily/infested with ticks ^{which are} therefore make it difficult to determine the most possible cause of the episode. The other abnormal constituents found in the urine cannot be specifically assessed because of the limited and unsystematic urinalysis undertaken in the study. Nevertheless, the presence of substances like protein and glucose indicate renal damage or excessive blood levels beyond the threshold of the kidney function. The autopsy findings, especially in guinea pigs, suggested renal damage on account of severe haemorrhage and enlargement. Lack of tangible explanation for the observed changes leaves room for speculation on the effects of intoxication that lead to necrosis and other degenerative tissue changes. The intoxication phenomenon could be supported by unresponsiveness in lymph nodes (van Rensburg, 1959) which I also found, progressive body wasting from no apparent cause despite adequate feeding, typical 'anthrax' septicaemia induced in guinea pigs.

Further evidence of toxicosis can be related to rapid onset of reaction and succumbing in guinea pigs to first infestation, development of non-infectious pyrexia and hypothermia (guinea pig). My clinical findings in the guinea pigs are in agreement with studies made by Neitz et al., (1969) and Howell et al., (1975) on the properties of salivary secretions of Ornithodoros savignyi. They demonstrated that effects attributed to the tick were caused by a toxin in the salivary secretion in addition to possible exsanguination. The toxic factors affecting body temperature response have been discussed before. Establishment of the role of intoxication in the general syndrome(s) caused by R. appendiculatus infestations would nevertheless require more refined salivary secretion administered to susceptible hosts. Single graded doses or multiple doses given over two to five days would avoid complications which are bound to arise from immune reactions if previously sensitized animals are used. Thus demonstration of specific toxicosis was not attempted because sufficient salivary secretion was not available for technical reasons and limited adult tick supply. Furthermore, the histological samples stored will not give/^aconsistent picture of the progressive tissue changes because of the random manner in which they were obtained. Death or end of trials were controlling factors and besides no autopsy was performed on the uninfested control group. The control calves had to be conserved because

of difficulty of rearing suitable animals for further experiments. Nonetheless, the evidence I have obtained from the primary and secondary experiments does strongly support incrimination of Rhipicephaline toxicosis for the disturbance induced in the health of the calves.

11.8. The sequel to presumed intoxication that culminated in extreme debility and prostration was death. Calves died after several repeated exposures whereas guinea pigs succumbed on first or second infestation. The calves were extremely emaciated and weak, indicative of deprivation which was not entirely consistent with the ration fed. The suboptimal blood constituents could not on their own culminate in death as the levels were not critical enough at the time of death. On the other hand the guinea pigs evinced rapid critical anaemia (extreme pallor, scanty watery blood, prostration), hypothermia or acute toxaemia. The intensity^{the} of haemorrhagic syndrome accompanying the toxaemia was frequently exaggerated during the secondary infestations. This was attributed to release of high levels of pharmacological agents, e.g. histamine or related compounds, from antigen-antibody reactions as demonstrated in experiment (VII.1) & (VII.3) (C.10.3 & 4). These agents provoke dilatation and leakage in capillary vessels (Tatchell & Moorhouse, 1968; Riek, 1955, 1962). It is possible that the response is augmented by a direct synergistic action of the salivary secretion (Tatchell & Binnington, 1973). Extravasation into tissues or exsanguination

reduce the oxidative processes in the body and, compounded by reduced venous return, would lead to anoxia. Anoxia invariably paralyses tissue activities, especially the brain, which is very vulnerable to low oxygen tension and glucose levels. Thus development of such a situation would accelerate the onset of death. However, low fluid intake during periods of extreme weakness in both species would cause dehydration. Consequently urine output is reduced, as occurred in guinea pigs, leading to accumulation of waste products, e.g. nitrogen waste products, ammonia, which are toxic in large amounts. These waste products can be further increased by the physiological breakdown of tissues due to pyrexia and cachexia (Coles, 1974). Besides, direct injury to renal tissue, as indicated before, does impair function, thus secondarily causing waste product accumulation. The changes produced in the blood biochemical equilibrium would have a major contributory role in the cause of death in the chronically infested hosts. Thus effects associated with death appear to act individually or synergistically but it can be deduced that the main causes are exsanguination and/or intoxication. Exsanguination mediated through massive R. appendiculatus feeding killed guinea pigs within few days of infestation. The calves on the other hand succumbed much later to repeated infestations of adult instar, having sustained severe debility and suboptimal blood changes. The debility could not be predominantly associated with

exsanguination since it occurred under immature instar infestations when blood changes were not seriously affected. Therefore death in calves could be predominantly attributed to intoxication though the mechanism underlying its effects cannot be precisely explained.

11.9. Autopsies revealed a number of changes which helped to explain the clinical manifestations that would otherwise remain obscure when considered on their own. Guinea pigs, for instance, showed oligouria/anuria which could not be attributed entirely to adipsia but the kidneys were found haemorrhagic and enlarged. Details of cellular changes in the nephrons are lacking since no histology was done. Depleted blood in the body tissue/vessels confirmed the clinical diagnosis of anaemia/exsanguination associated with pallor in the skin and visible mucosae in the guinea pigs. Clinical assessment of loss in body condition was confirmed by appearance of dropsical degeneration in various tissues - heart, kidneys, wasted muscles, absolute loss of fat. Dropsical changes under/^{the}skin, which could be related to hypoproteinaemia, were not detected clinically most times. Similarly, exudate/transudates in serous cavities, whilst in excessive amounts can disturb the normal function of organs, were only revealed at autopsy. The appearance of such accumulations could indicate intoxication affecting

blood capillaries or cachexia. Lymph node changes, except in guinea pigs, were confined to local drainage nodes observed clinically, thus demonstrating they were related to specific effects of the tick secretions. This is very relevant to field diagnosis of conditions involving lymphadenopathy which sometimes may be localised in nature. However, guinea pigs presented a much wider involvement of the lymph nodes which could be attributed to body size and high susceptibility to tick effects leading to widespread vascular disturbance. Furthermore autopsy revealed the vivid disruption of circulatory function in the guinea pigs, especially on repeated infestation which was not so evident clinically. Haematological estimations revealed regenerative features and these were supported by finding active state of the bone marrow in calves. Difference in response to tick effects attributed to host species differences was revealed in the appearance of the spleen. The calves had a hypoplastic organ whereas the guinea pigs showed a red thick spleen. In the guinea pigs, and to lesser extent in calves, persistent bleeding from tick wounds was supported by finding chicken fat clots or uncoagulated blood many hours after death if the autopsy was delayed. This showed that whatever caused bleeding from the wounds was circulating in the body. In this regard it can be suggested that the anticoagulant occurs in the salivary secretion of R. appendiculatus or it is liberated from complexes of immune responses (Tatchell,

1969a). However, Code (1939) found that high levels of histamine in the serum of immune animals impaired the coagulation mechanism. Thus delayed coagulation observed in guinea pigs infested more than once can be partly attributed to histamine release.

11.10. The secondary experiments based on null hypothesis were undertaken to elucidate some of the observations made in the primary experiments. Primary experiments presented response which varied in pattern, onset and severity and marked differences were found between first and subsequent exposure responses. The investigations of the secondary experiments revealed differences in responses of calves and guinea pigs to tick agent(s) effects. Thus guinea pigs sustained critical blood changes from single massive infestations, whereas the calves showed such changes after several infestations. Nonetheless, allowance should be given for the density of exposure relative to host size. The secondary experiments confirmed a number of the selected features and further demonstrated responses not clearly revealed during primary experiments. The investigations also involved establishing whether no significant differences existed between responses to first and repeated exposures. This differentiation is relevant to studies requiring susceptible hosts when their source includes a mixture of animals with different levels of resistance to tick effects. Conversely a situation may arise when immune

animals are required for biological studies on tick resistance. Thus hypothesis (IV), which states, 'There is no difference between the effects induced by primary and repeated exposures to the agents' was applied to results from primary experiments. The hypothesis revealed clear differences though in some features there were no obvious distinctions. Several workers using different tick-host systems have demonstrated clinical (Hewetson & Nolan, 1968, Hewetson, 1971, 1972; Riek, 1962, 1956; Allen & Wikel, 1978), histological (Tatchell & Moorhouse, 1968; Schleger et al., 1976; Wikel & Allen, 1978) and tick performance (Roberts 1968b,c; Allen, 1973; Trager, 1939a; Koudstaal et al., 1978; Branagan, 1974) differences between primary (first) and repeated exposures. The immune responses, which significantly influence the reactions, have been investigated with ^{the} aim of determining the levels of resistance to tick challenge. In this respect studies have been made where cattle were exposed for protracted periods to find whether challenge boosted the resistance. This was basically assessed by tick performance. However, the responses were not consistent as differences were complicated by the appearance of significant variation between breeds and individuals (Tatchell & Moorhouse, 1968; Francis, 1966; Howell, 1966; Riek, 1956, 1962; van Rensburg, 1959). This phenomenon could partly explain the different reactions in my calves which comprised various Bos taurus breeds.

They showed notable differences in clinical manifestations to R. appendiculatus but the response in the few jerseys did not differ markedly from the other breeds (Friesian, Ayrshire). I found also that the responses to the exposures of individual instars showed varied severity. Generally the adults inflicted the most severe reactions and the larvae the least. However, the larvae provoked/^{the}most severe hypersensitivity reaction which predominantly manifested pseudovesicles. The small numbers of animals used do not permit drawing specific conclusions on differences in responses attributed to breeds. Nonetheless, individual animals varied markedly in their responses, from virtually no frank skin reaction to excessive bleb formation and exudation. In analogy the guinea pigs reacted differently from calves as stated above. The peculiarities of the guinea pig, e.g. high sensitivity, account for these differences and have led to varied assessment on their suitability for tick studies. Riek (1959) found them unsuitable for B. microplus experiments but a lot of other tick studies have adopted the species as the standard host (Allen, 1973, 1977; Wikel & Allen, 1978; Bagnall & Rothwell, 1974). Thus allowing for differences between breeds, host species and individuals the hypothesis revealed similarities and distinct variations in the responses to the R. appendiculatus agent(s) in the calves and guinea pigs. The parameters which showed distinct variations included hypersensitivity state,

pruritis, pyrexia (mono and diphasic), erythema/congestion, oedema and haemorrhage. In view of these observations the hypothesis was not wholly false. Reappraisal of these clinical parameters does not clearly reveal any one response that can be significantly correlated with levels of resistance to tick effects because of the difference in response described above. Nonetheless, the cutaneous hypersensitivity test could provide fairly reliable measure.

11.11. Extreme debility and death attributable to tick infestation have been reported previously (Francis, 1960; Little, 1963; Clifford, 1954; Seebeck et al., 1971; Macleod, 1933, Johnston & Haydock, 1969) and identical effects have been demonstrated presently using Rhipicephalus appendiculatus ticks. Philip et al., (1935) found that cessation of infestation (deticking) before animals became extremely affected was followed by rapid and complete recovery. Similar recovery has been observed in the present study when infestation ceased. One animal which was virtually dying of critical anaemia, extreme wasting and weakness, but still able to feed, showed^a dramatic recovery. Within four weeks the blood picture and body condition were very much improved and the calf became very active. The rapid recovery shows that the tick effects, though severe,

did not completely paralyse the regenerative potential of the body. This observation was supported by finding that the bone marrow of the succumbed calves still showed evidence of haemopoietic activity which was confirmed by the appearance of circulating reticulocytes. Nonetheless, Riek (1957b) and Seebeck et al., (1971) observed that chronically heavily infested animals had long convalescence despite adequate feeding. The slow recovery was attributed to persistent toxicosis. A striking feature in the responses of my calves was the sustained appetite throughout the repeated infestation in the absence of secondary complications. Persistence of the appetite supported by adequate ration should maintain body condition but this was not the case during infestation. However, on cessation of infestation the sustained appetite and adequate ration would give a tremendous boost to the recovery rate. Under field conditions it is not unusual for animals to lack minerals in their feed. This deficiency could be of soil/pasture origin or as a result of unbalanced ration. Removal of blood by feeding ticks deprives the animals of minerals e.g. iron, cobalt, copper, calcium, potassium, phosphorus etc. which are essential for normal body metabolism. For instance, iron and copper deficiency would retard blood formation and growth or deficiency of calcium and potassium would affect muscle activity. It is possible that the speed of ^{the} recovery is influenced by/levels of these essential electrolytes. Thus the calves in my study were given a

a constant balanced mineral ration. In view of this observation it may be relevant to monitor the electrolyte changes in the blood of infested animals. This should differentiate other possible contributory factors.

11.12. In conclusion this study has demonstrated that repeated infestations of R. appendiculatus applied artificially can adversely affect the health of cattle. All three instars individually or concurrently applied are harmful but the infestations of adults and concurrent three instars inflicted the most severe effects. The clinical-pathological manifestations induced included pyrexia of varied degree; circulatory derangement, e.g. oedema, anaemia and haemorrhage; lymphadenopathy; dermatitis - eczema, nodules accompanied by alopecia; hypersensitivity reactions; and intense pruritis. The infestations induced immune responses demonstrable by allergic tests. The significant role of histamine in the mediation of immune responses was demonstrated by administration of antihistamines. In severely debilitated animals the induced syndrome(s) culminated in death in both primary (guinea pigs) and repeated exposures. Besides the direct effects, the tick bite wounds predisposed the animals to secondary bacterial infection manifested as lymph node abscesses and associated chronic bronchopneumonia. Both primary and repeated infestations induced significant responses but the repeated exposures generally produced different

and more severe reactions. Significant differences were demonstrated between groups in the haematological and biochemical estimations. Death was attributed to exsanguination, debility and intoxication and these observations were supported by autopsy findings. Debility was associated with anaemic changes and pyrexia reaction and subtle effects of tick secretion.

The secondary experiments revealed further evidence in support of intoxication and immune response effects. Pyrexia of non-infectious origin was induced under antibiotic medication administered to animals which were free from tick-borne diseases. The appearance of the pyrexia during the tick feeding period indicates that the injected salivary secretions was pyrogenic. The repeated exposures induced a diphasic pyrexia, but the first pyrexia rise was attributed to hypersensitivity reactions (Aitkin, 1960; Wagland, 1975). Conversely, hypothermia was induced in guinea pigs during primary (first) exposure to massive infestation of R. appendiculatus. This was attributed to paralysis of the heat regulating centres by specific effects of the tick secretions. Such paralysis was not accompanied by shivering in the affected guinea pigs. Moderate to severe circulatory changes related to dilatation and increased permeability of capillaries were produced in both primary and repeated exposures. The changes were very vivid in the guinea pigs. The reactions were attributed to direct tick effects (intoxication) during

both exposures and during the secondary exposure to immune effects. Suspension of digestive functions in the primary exposure in guinea pigs which succumbed while still in good condition strongly implicated direct tick effects. Progressive wasting despite adequate feeding and in the absence of significant systemic clinical reaction (pyrexia) culminating in extreme debility and death could be attributed to specific tick effects (toxicosis). Hypoplasia and the firm texture of lymph nodes and splenic atrophy were similarly related to direct tick effects. Early death during primary exposure in guinea pigs protected with antibiotics was attributable to toxic tick secretions. However, death during repeated exposures was due to concurrent toxic and immune effects, thus administration of antihistamines did not offer complete protection in some infested animals. Nonetheless, antihistamine administration demonstrated that histamine played a major role in the mediation of immune responses. This role was vividly illustrated in the massive inflammatory changes (erythema-oedema) in infested sites which were partly inhibited by antihistamine therapy. Besides, therapy facilitated successful tick performance where it had failed before. Immune response to R. appendiculatus effects developed within fourteen days of first exposure and the allergic tests evinced moderate to severe local reaction. The severe local reaction simulated an Arthus reaction that culminated in haemorrhage,

exudation, necrosis and sloughing. Massive clustering during primary exposure caused severe wounding, exudation and necrosis at the infested sites. Clinically the hypersensitivity state caused severe bleb formation (pseudovesicles) that left 'pox virus' mark scars in some animals. Furthermore, the effects of immune responses induced by R. appendiculatus agent(s) were passively transmissible by immune serum which demonstrated that part of the response comes from humoral antibodies. Thus the adverse effects on the health of the animals were mediated through intoxication, immune responses, subtle causes of debility and deprivation, in addition to exsanguination.

The immune reactions inhibited tick performance, particularly the larvae. The tick rejection was mediated through frank and inapparent epidermal reactions. The influence of some of the immune responses upon tick viability are not clear. The host influence on the tick performance is thus advantageous in the control of their population when ^ahypersensitivity reaction virtually kills all the attached larvae.

Death and debility, besides transmitted diseases, caused by Rhipicephalus appendiculatus infestation pose serious economic problems in cattle production. These problems involve reduced milk and beef production, retarded growth rates in immature stock, reduced value of infested skin/hide and secondary infections. Abundance of tick population strains the resources for cleansing

and other measures directed toward control of infestations. Nonetheless, it takes very few infected ticks to establish tick-borne disease. Therefore a break in the life cycle of the stages capable of transmitting the disease(s) will be important. Justification of the preventive measures would require full knowledge of the tick physiology, ecology and its effect on the host. Thus there is need for further investigation of the physiological and biochemical effects induced in the host and their histological relationship in the internal organs. Such a study would go a long way to meet the recommendations made by Neitz (1962) regarding the seriousness of toxicosis from Rhipicephalus appendiculatus infestation. This study should also cover the composition of the saliva as it affects susceptible hosts. The effects of refined salivary secretion should be related to direct responses to low and high concentration doses. The investigation would examine such features as local (cutaneous) and systemic reactions monitored daily. The systemic examination should include the circulation and blood composition, lymphatics, vital functions of different organs (liver, kidney) relative to clinical manifestations. Since part of the Rhipicephaline syndrome is mediated through immune effects then the development of resistance to tick secretion should also be investigated in hosts repeatedly exposed. The investigations should examine^{the}/minimum dosage (exposure) to produce demonstrable

effects and the duration of such effects. The possibility of an immunisation against R. appendiculatus instars should also be examined, especially aimed at the larvae which appeared to be the most vulnerable to host reactions.

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